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Jordan-Burrows Textbook of
BACTERIOLOGY

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PREFACE

Twenty years ago it was freely predicted by a number of eminent workers that a new era of bacteriology was beginning which would be characterized by a greater emphasis upon the nature of these microorganisms, and especially their biochemical physiology. This has been more than amply confirmed in recent years, and it is becoming increasingly clear that bacteriology has largely emerged from a naturalist period of essentially empirical observation into an analytical, quantitative phase in which the proof of specific bacterial etiology of infectious disease, for example, has been extended into analysis of the functional mechanisms of pathogenesis. The changing approach has given great promise, and the extent to which a book of this kind becomes appreciably obsolete within a few short years is impressive evidence of the progress that has resulted from it. Most encouraging is the extent to which the various applied branches of bacteriology are drawing together on a common fundamental basis, and the relating of these forms to other organisms through basic physiological processes, such as respiration, nutrition and genetic control.

The pattern is reflected in the development of this book through successive editions, its basic premise has been the assumption that the sound pedagogical approach is that of an understanding of fundamental principles out of which application flows. There are few better illustrations of the importance of apparently impractical "pure" research than the elucidation of the nutritional requirements of bacteria, with subsequent development of a rational theory of chemotherapy. This area of research further points up the significance of the anabolic phase of bacterial metabolism as accumulating evidence suggests more and more strongly that the point of attack of certain of the antibacterial drugs is in the reactions of synthesis. In this same vein, the apparently academic question of the relative importance of, on the one hand, selection of chance mutants, and, on the other, a mass action basis of adaptive enzyme formation in the development of drug-fast strains of pathogenic bacteria may, perhaps, prove to be of considerable significance through mutually exclusive adaptations. Considerations such as these tend to reinforce belief in the validity of the premise, and it continues as the basis of the present edition.

There has been, necessarily, a considerable amount of rewriting which has ranged in extent from entire chapters to sections, together with appropriate modification of innumerable minor points. One change in order of presentation has been made with a shift of the chapter on bacterial physiology so that it precedes that on physical and chemical agents.

The chapter on laboratory methods has been completely rewritten in a much more specific form. It is in no way intended to be exhaustive, but rather to include only the majority of the common laboratory procedures which the student will use, and to make available to him specific information concerning

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them. Many of these are, of course, amplified and extended in subsequent sections on the bacteriological diagnosis of the various infectious diseases. In the preparation of this material the writer has drawn heavily on the *Manual of Methods of the Society of American Bacteriologists* and on *Diagnostic Procedures and Reagents* of the American Public Health Association in an effort toward further standardization of laboratory procedure at this basic level. The chapter on streptococci has also been completely rewritten with closer integration of these bacteria as a group of pathogens, and a relating of the various clinical forms of infection on this common basis. The rapid advances of the past few years in bacterial genetics have necessitated a reconsideration of bacterial variation, and this chapter has likewise been entirely rewritten in a somewhat different form with consideration of the observed variations, the relation of these to one another, and finally the questions of underlying mechanisms. In conjunction with this, the earlier section on the effects of radiation has also been rewritten.

A considerable number of sections have also been rewritten. These include the sections on respiration and carbohydrate metabolism, in which greater emphasis has been placed on the cyclic nature of these processes together with integration of the modes of formation of the products of carbohydrate metabolism as a generalization of pyruvate metabolism. Sections on the biosynthesis of carbohydrate and amino acids have also been added, and the catalysis of these and the catabolic phases of metabolism related to nutritive requirements. Similarly, the discussion of the mechanisms of antibacterial action has been rewritten and extended, and related more closely to bacterial physiology. The section on hypersensitivity has been revised also, with correlation of clinical disease with basic immunological phenomena, and entirely new sections on *Donovania*, rickettsialpox and infectious hepatitis have been added.

Other more general changes include the addition of a considerable number of electron micrographs, and more recent data on the seasonal incidence of infectious disease. As to the former, only relatively recently has resolution been such that electron microscopy has contributed to and become a valuable adjunct in the study of bacterial morphology. Its value is apparent in the demonstration of intracellular structures perhaps identical with nucleus-like chromatinic bodies, the convincing evidence of the origin and insertion of flagella, the elucidation of the details of L type reproduction, and other hitherto obscure problems. Electron micrographs have, therefore, been freely used to illustrate these and other matters, but have not been substituted for photomicrographs which remain, of course, invaluable to the student.

The writer is greatly indebted to his collaborators, Dr. F. B. Gordon who is responsible for the viruses, Dr. R. J. Porter who has continued his responsibility for medical parasitology, and Dr. J. W. Moulder who, with this edition, has taken over the general subject of bacterial physiology and has written the sections on respiration, carbohydrate metabolism and synthesis. These contributions are now recognized on the new title page coincident with the dropping of the name of Dr. E. O. Jordan as co-author. With the rewriting of this book through successive editions since Dr. Jordan's death thirteen years ago, his connection with it has become so tenuous that his name can no longer

The phenomena that present themselves for study are derived from the most diverse sources. Some come to us from epidemiology, using that term in its widest sense. Under natural conditions different animal species show a widely differing incidence of certain infections. Thus, anthrax is in the main a disease of herbivora. Of the animals dying of anthrax in this country in 1914, 733 were cattle, 5 were sheep, 32 were swine and 25 were horses. In Australia and South America sheep are more commonly affected; but Algerian sheep are stated to be highly resistant. Tuberculosis is one of the commonest natural infections of man and cattle. It is common in pigs and in fowls. It is relatively uncommon in sheep, goats, horses, cats and dogs; and very uncommon as a natural disease in rabbits, guinea-pigs, hamsters, rats and mice. Among mankind there are well-marked racial differences in its incidence and severity. In eight of the great cities of the United States in 1920 the mortality from tuberculosis among the white population varied from 0.794 to 1.216 per 1,000 living; among the coloured population it varied from 2.855 to 4.205 per 1,000. Such instances could be multiplied *ad nauseam*.

The epidemiologist also records differences in the incidence and fatality of various infective diseases at different ages, suggesting in many cases an increase in resistance with age. He notes also that repeated attacks of the same infective diseases are in some instances very rare, as in measles, or smallpox, or diphtheria, or typhoid fever, whereas in others they are relatively common, as in influenza, or pneumonia, or the common cold.

Other data come to us from clinical medicine in the narrower sense. Infections that, in their usual course, progress slowly to death or recovery sometimes assume a fulminating form. Such a protean infection as tuberculosis shows the greatest diversity in the varying prominence of its local and general manifestations. In any infective disease we are confronted with a wide variety of circumstances in which some patients die and some recover.

For the immunologist these are crude facts that require analysis. Using his own methods he re-examines the phenomena presented by natural infection. Sometimes he transforms the picture they present. He finds, for instance, that the recorded frequency of natural tuberculosis in various animal species does not in all cases reflect their relative resistance to experimental infection (see Chapter 59). He discovers also that there may be a diversity of immunological states within a single clinical syndrome. This is true of tuberculosis; there are different types of the tubercle bacillus, and a given host species is more resistant to one type than to another. It is true of lobar pneumonia. For the immunologist this is not one disease but several, each caused by a significantly different type of pneumococcus; and he notes that the statement that second attacks of pneumonia are not uncommon may belong to the Baconian category of a truth that has in it a mixture of a lie. Again he finds that enteric fever is not one but many; and his attempts at interference are planned accordingly.

Another important change that the immunologist makes in the clinical and epidemiological picture is in regard to the character and extent of the association between any given parasite and the host species that it infects. He finds that the real range of interaction includes states of equilibrium in which the host shows no overt signs of disease. Whether we call all these conditions latent infections, or refer to many of the hosts as healthy carriers, matters little. No hair-splitting definition will help us much. The significant thing we have learned is that in some infections, such as measles, contact between a previously uninfected host

be asked to carry the burden of responsibility for authorship, but is retained in the title as a token of the writer's deep affection and respect.

The number of colleagues who have taken the time and trouble to offer suggestions and criticisms is very great indeed. The writer's obligation to them all can hardly be overestimated, and he hopes that they will continue to offer such invaluable advice. Of these, the writer is especially indebted to Dr. Stuart Mudd and his co-workers for advice and assistance regarding electron micrographs and newer studies of bacterial morphology, to Dr. C. Robinow for photomicrographs of his chromatinic bodies, to Dr. Robinow and Miss Woudera van Iterson for electron micrographs of flagella, to Dr. R. W. G. Wyckoff for his superb micrographs showing phage generation, and to Dr. W. A. Jamieson who allowed the writer free access to the entire collection of electron micrographs of the Lilly Research Laboratories. Last, and by no means least, the writer is deeply appreciative of the help of Mrs. Gertrude Conklin and that of the staff of the W. B. Saunders Company in carrying this book through the press to its final form.

August, 1949

WILLIAM BURROWS

the existence of an indeterminate borderline class, he is justified in using the classification, because most of the time the distinction can be usefully made.

And so, if he has a taste for classification, the immunologist can draw up some such list as this:

1. Innate Immunity (i) Non-specific.
 (ii) Specific.
2. Acquired Immunity (i) Non-specific.
 (ii) Specific.
 - (a) Active.
 - (α) Naturally acquired.
 - (β) Artificially induced.
 - (b) Passive.
 - (α) Naturally acquired (congenital).
 - (β) Artificially induced.

Innate, or Genetic, Immunity.

Of innate as opposed to acquired immunity, we have noted that different animal species may display wide differences in their resistance to various bacterial parasites, or to their toxins. This species immunity is of great practical importance in relation to the communicability of infective disease from animals to man, or from one animal species to another.

There can be no doubt that differences in innate resistance also occur within any animal species, one individual differing from another in this biological character as in any other. Of the extent of these differences and of the laws that govern their inheritance we as yet know very little. It is obviously difficult to obtain unequivocal evidence in man for the inheritance of susceptibility to a given infection from field studies of human populations. Nevertheless, the evidence is highly suggestive in some infections, for example, in leprosy (Aycock 1941), poliomyelitis (Aycock 1942), tuberculosis (Chapter 58), and rheumatic fever (Chapter 68). A number of workers have studied this problem by direct experiment, and their results indicate that it is possible to increase or lower the average resistance of a given strain of rats, mice or other experimental animals, by selective breeding. (See for instance Rich 1923, Webster 1923, 1924*a, b*, 1925, 1933*a, b*, Pritchett 1925, 1926*a, b*, Lambert and Knox 1928, Irwin 1929, 1933, Irwin and Hughes 1931, 1933, Lambert 1932, Schott 1932, Gowen 1933, Gowen and Schott 1933*a, b, c*, Schütze *et al.* 1936, Hill *et al.*, 1940, Lurie 1941, Gowen and Calhoun 1943.)

Experiments of this type are, however, subject to great technical difficulties. The obvious method of obtaining a strain of animals with a high genetic resistance is to infect an adequate sample with the bacterium under study, breed from the survivors, and repeat the process through several subsequent generations. This plan has, in fact, been followed by some of the workers referred to above; but it is clearly open to serious sources of error. In testing the resistance of our original generation we shall certainly alter it, and the effect of this alteration will not be confined to the parent animals. The surviving females will pass on a temporary passive immunity to their young; and, since most of the species commonly employed in such tests attain sexual maturity within a few months at most, this congenital passive immunity may persist until the F_1 generation are tested. A much

We are still largely ignorant of the defence mechanisms whose effectiveness is determined genetically. Gorer and Schütze (1938) found some evidence that in a strain of mice resistant to *Salm. typhi-murium* the specific antibody response to the H antigen was better than in a susceptible strain, but this relation did not hold for O antigen, nor for the H or O antigens of *Salm. enteritidis*. Lurie (1941), on the other hand, working with inbred strains of rabbits, of low and high resistance, demonstrated that both the degree of cellular response to invasion by the bovine tubercle bacillus, and of skin response to the injection of heat-killed bacilli, was higher in the tuberculosis-resistant strains (see p. 1490). A similar, and more obvious rapid development of the cellular reactions, and to some extent of antibody response, was observed when the same strains of rabbit were tested by inhalation of a less rabbit-virulent human strain of tubercle bacillus (Lurie *et al.*, 1952). In inbred strains of mice selected for resistance to *Salm. typhi-murium* the degree of resistance was found by Gowen and Calhoun (1943) to be correlated with the mean number of leucocytes circulating in the blood. A large number of circulating leucocytes, however, is probably a manifestation of a general not of a specific resistance to the organism, for as Reich and Dunning (1941) showed in rats, the numbers of leucocytes may be correlated with general fitness for survival.

Severens, Roberts and Card (1944) studied the defence mechanism in two breeds of hen, which as chicks aged 1-10 days were respectively susceptible and resistant to infection with *Salm. pullorum*. All the demonstrable differences in the reactions of the two breeds were connected with lymphocytes. Age for age, the number of circulating lymphocytes, the rate of their normal increase after hatching, and the number present in the tissues of the spleen, were all greater in the resistant breed of chick. Moreover, splenectomy reduced both resistance and the circulating lymphocytes in the resistant chicks, but had little effect on the susceptible. It is suggested that the greater availability of lymphocytes may have increased the resistance by providing large numbers of precursors for macrophages in the tissues of the resistant chicks.

There may also be genetic differences in the ability of phagocytes to digest the pathogen. The splenic reticulo-endothelial cells of mice resistant to *Salm. typhi-murium* digest the invader rapidly, and those of susceptible mice digest it very little; moreover, whereas the salmonella inflicts metabolic damage on the whole liver in the susceptible strain, in the resistant it produces a few severe lesions in an otherwise healthy liver (Oakberg 1946). In selected strains of fowls tested with *Salm. gallinarum*, Bell (1949) recorded an association of resistance with the ability of the circulating polymorphonuclear leucocytes to digest the salmonella *in vitro*. He also noted a slightly higher body temperature, and greater capacity to maintain the body temperature when subject to low air temperatures, in the more resistant strains. Weir (1949) recorded in mice an association of higher mean blood pH with resistance to *Salm. typhi-murium* (of the order of 7.4, as against 7.33 in susceptible) both in mouse strains as a whole, and in the individuals of populations resulting from crosses between resistants and susceptibles. But when strains were bred for high and low leucocyte counts, and high and low pH respectively, it was found that mice with low leucocyte counts and low pH were the most resistant (Weir *et al.* 1953). If it is assumed that blood leucocytes and pH do in fact contribute to defence against infection, then resistance depends on complex interactions of characters which, taken independently, do not necessarily contribute to resistance. Again, if we assume that intoxication by bacillary

and repeated the procedure for ten generations. The strain was resistant to the toxin as compared with control unselected mice, but in spite of this it had not acquired any resistance to infection with the living bacillus.

In summary, then, we have undoubted examples of heritable resistance to certain experimental infections; resistance which by breeding can be selected without exposure to the infection in question, and which in some cases has proved, in comparisons of

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less frequent, local lesions common and relatively extensive, and latent infections increasing in frequency.

With partial immunity of a high grade, death no longer occurs, bacteraemia is infrequent and, when it occurs, is slight and transient. Local lesions are becoming much less frequent and, when they occur, much less extensive. Latent infections reach a maximum frequency and then begin to decline.

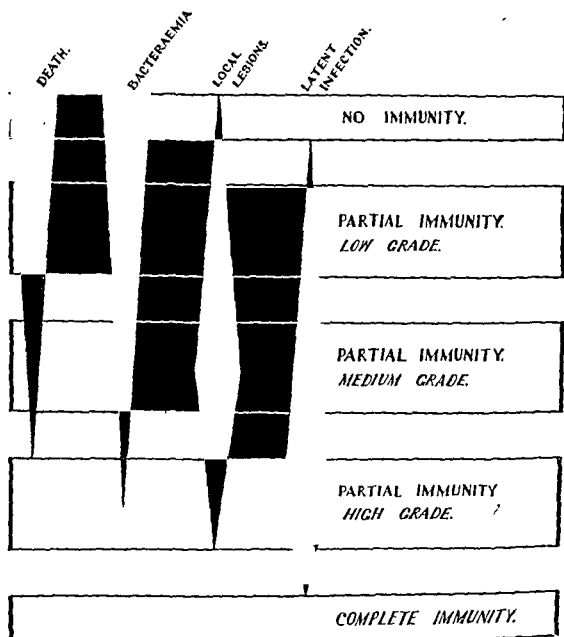


FIG. 239.

Finally we reach the limit—perhaps never fully attained—of complete or solid immunity. The host is entirely impervious to all attacks of the parasite.

It will not have escaped attention that the grades of resistance that we have labelled as partial immunity are compatible with severe and often fatal infections, and that many infective diseases in their common clinical form might be regarded as occurring in partially immune persons. This view is almost certainly the right one. The syndromes that normally characterize such diseases as typhoid fever,

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will learn something from their success or their failure. In medicine this is not always the case, simply because the practice of medicine is a much more difficult thing than any kind of mechanical industry, or indeed than any industrial process at all.

It may be quite easy to tell when an immunological procedure is an unqualified practical success. It is much more difficult to tell whether it is a partial success, or an unqualified failure. And this cause of confusion, which in truth affects the immunologist in the laboratory almost as much as the physician in the ward, is so important that it deserves a separate chapter.

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will learn something from their success or their failure. In medicine this is not always the case, simply because the practice of medicine is a much more difficult thing than any kind of mechanical industry, or indeed than any industrial process at all.

It may be quite easy to tell when an immunological procedure is an unqualified practical success. It is much more difficult to tell whether it is a partial success, or an unqualified failure. And this cause of confusion, which in truth affects the immunologist in the laboratory almost as much as the physician in the ward, is so important that it deserves a separate chapter.

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here to a brief discussion of some general principles, with illustrative examples drawn from a few immunological problems.

The cause of our troubles is sufficiently obvious. It was set out very clearly by Greenwood (1921) and by Yule (1921). The worker in the more exact sciences can reduce his uncontrollable variables to a small and often to a negligible residuum, and can so obtain constantly reproducible results. We cannot. In experiments, on living animals, and still more in assessing the value of therapeutic or prophylactic procedures in man, we cannot exclude the interplay of factors about which we know little, except that they are certainly very numerous and may be very important. We cannot avoid an element of randomness in our observations; but, if we have planned our experiments wisely and fortune has been kind in eliminating major disturbing factors that we could not have foreseen, this randomness will be of the sort which we can express in numerical values and whose effects can therefore be assessed with some accuracy in the interpretation of our results.

It will simplify discussion to consider in turn a series of problems of the kind that are continually presenting themselves for solution.

THE MEASUREMENT OF RESISTANCE

To take the most obvious problem, though not the simplest, how can we measure the resistance of an animal to a particular parasite or to its toxin? Suppose first that we wish to measure directly the resistance of the animal as a whole, taking death or survival as our test. Then it is only under quite exceptional conditions that we can obtain *any* measurement on a single animal. If we are dealing with a very powerful toxin that kills rapidly, within a few minutes or hours, it is sometimes possible to give repeated and increasing doses at short intervals and so to measure approximately the amount which is necessary to cause death. But if our toxin acts more slowly, or if we are dealing with a living bacterium, this method cannot be employed. If we start with a small dose and the animal fails to die during the period—usually measured in days—within which we should expect death to occur, we cannot test it again by giving a larger dose, because our first dose will almost certainly have changed its resistance. It is not, from our present point of view, the same animal as before. If we start with a large dose and the animal dies, we do not know how it would have reacted to a smaller dose; if it lives, we know that it is resistant at that particular dose level, but we do not know and can never discover how it would have reacted to doses larger still. It is clear that, if we take death or survival as our test, we must deal with groups not with individuals. This condition is, in truth, usually implicit in the question we want to ask. We are not often interested in the resistance of a particular mouse, or guinea-pig, or rabbit, as such. We are almost always regarding the individual as a representative of a class—normal, or treated in some way that may, we think, have altered resistance to a particular bacterium or toxin—and few of us are naïve enough to believe in a standard guinea-pig, even if we confine ourselves to animals of a given breed, sex, age and weight. What we sometimes fail to realize is that we must, in most cases, test large numbers of animals before we can regard our result as representing truly the average behaviour of the class from which those animals are drawn.

There is another problem, intimately and necessarily bound up with that of measuring resistance, namely, the problem of measuring, or at least specifying,

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to our treated animals a large dose of bacteria or toxin, which experience has shown to be certainly fatal to all untreated animals of the same species, age, weight, etc., and note that the treated animals survive. But we shall be wise to make very sure about our "certainly fatal" dose, especially if our group of treated animals is small. We shall, indeed, always test a few untreated controls to guard against any gross experimental error.

But the increase in resistance may not be of this dramatic kind. Our treated animals may be unable to withstand a dose of bacteria or toxin that will kill all untreated controls, though the mortality in treated and untreated groups may differ appreciably. Our problem is to determine whether this observed difference is likely to have occurred as the result of chance.

In the following section, we discuss relatively simple examples and assume that the experimental animals behave according to expectations based on certain statistical arguments. Reference to modern text-books of statistics will show that we might have used other statistical concepts—and indeed, for more complex examples we should have had to do so. But our examples are sufficient to illustrate the mode of statistical argument, and to introduce the reader to some of the elementary terminology.

Suppose that we know, from an extended experience, that a particular experimental procedure causes an average death-rate p in a large group of animals. This rate, p , expressed as a fraction of unity (e.g. $p = 0.5$ for a 50 per cent., and 0.3 for a 30 per cent. death-rate), can also be taken as the probability of one animal dying. The probability of its living is $(1 - p) = q$; and q clearly is the survival rate among the animals, i.e. if the percentage mortality is 30, the percentage survival must be 70.

Supposing we know the true average mortality to be 0.5, how often are we likely, in a single test, to observe it? Two animals A and B are injected. The probability of A's dying is p and of B's dying is p , and of both dying is p^2 . The probability of both living is q^2 . The other possible results are (i) A's dying and B's living and (ii) B's living and A's dying; and in both cases the probability of the event is $p \times q$. The probability of one animal dying and the other living, irrespective of which does which, is $2pq$. We have therefore:

Probability of two deaths	p^2
Probability of one death	$2pq$
Probability of no death	q^2

These are the successive terms of the expansion of the expression $(p + q)^n$, where $n = 2$, and we obtain the results for larger groups of n animals by substituting the appropriate value for n in the expression. For instance, with four animals, the probability of getting 4, 3, 2, 1 or 0 deaths in any single test would be p^4 , $4p^3q$, $6p^2q^2$, $4pq^3$ and p^4 respectively. Both p and q are 0.5, so that these five terms are respectively 0.0625, 0.25, 0.375, 0.25, 0.0625, and represent the relative frequencies of each of the 5 possible events. They are plotted in Fig. 240A. The curves for B and C take us two steps further, being the relative frequencies when $n = 8$ and $n = 12$. As n increases, the figure becomes less step-like, and shows a decreasing proportion of its total area at each end of the base line. These figures represent distributions of the relative frequencies of the various possible events. The first important fact about a frequency distribution is the average, or mean, frequency, which is used to characterize the distribution. In A, B and C the mean frequency is in each case $n \times 0.5$, i.e. 2, 4 and 6 deaths respectively. The other frequencies are symmetrically disposed on either side of the mean. (The symmetrical disposition is the result of taking $p = q$. If we take $p = 0.3$, and therefore $q = 0.7$, the relative frequencies of 4, 3, 2, 1 and 0 deaths in a batch of 4 mice are given by the binomial expansion of $(0.3 + 0.7)^4$

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that the 3 groups in A, B and C are not completely alike, even though all the animals are, by hypothesis, equally susceptible to the lethal agent. In A, for example, the frequency with which a deviation of ± 50 per cent. from the mean will occur by chance is p^4 , which is 0.0625 or about 6 times in 100 trials; whereas in C, the same deviation will occur by chance about twice in 10,000 trials, since p^{12} is 0.000244. More generally, as the relative heights of the columns in A and C show, the bigger deviations from the mean are less likely to occur in the test with the larger number of animals; and there is a greater probability of obtaining results near the expected mean death rate of 50 per cent. In comparing distributions, then, we must consider not only the mean, but also the range over which the deviations from the mean are spread; to do this we need a measure of the spread of deviations applicable to all distributions of this type. This measure we find in a value known as the standard deviation, which is the second important characteristic of a distribution.

For the better appreciation of the standard deviation, we shall consider a distribution that is more general in form than A, B or C. These are step-like collections of parallelograms, the area of each of which corresponds to the relative frequency of each event. If we make n very large, say 1,000, and reduce the scale upon which we record the events, the figure for $(p + q)^{1000}$ approximates to a continuous curve of the form D. This curve is known as the *normal curve*. The distribution of frequencies of a number of biological

TABLE 59

Deviation in Terms of the Standard Error; or Ratio between a Difference and its Standard Deviation.	Odds against this Deviation or Difference being the Result of Random Chance.
0.5	0.6 to 1
1.0	2.15 to 1
1.5	6.5 to 1
2.0	20.98 to 1
2.5	79.53 to 1
3.0	369.4 to 1
3.5	2,149 to 1
4.0	15,773 to 1
4.5	147,188 to 1

measurements, such as the height of human beings of a certain age, or the resistance of a certain type of animal to doses of a certain poison, approximates to this mathematically defined curve. In many of the measurements we make in the study of immunity, the deviations from mean values can be fitted to a normal curve, though not necessarily in the form of our raw data. The abscissae in Fig. 240, it happens, are on a linear scale. Normal distributions of this kind are not commonly found in nature; but, by suitable transformation of the variate that we measure into a logarithmic or other scale, we obtain a sufficient approximation to the normal curve for valid statistical calculations (see Gaddum 1945). For reasons that will be found in the statistical text-books, we may assume with some confidence that in the measurements of resistance, etc., with which we are mainly concerned in immunity, the deviations from a mean value will be distributed much as they are in the normal curve, and that the arithmetic of the normal curve will be applicable to most of our data.

The standard deviation of a distribution that approximates to the normal may be obtained arithmetically, by squaring all the deviations from the mean, adding the squares together, dividing the result by the total number of observations, and taking the square root. The resulting figure, the "root mean square deviation," is usually written as σ . It has a positive and a negative value and is correspondingly measured to the right and left of the mean in graphic representation of the distribution. In Fig. 240 D, for example, the base line has been marked off at $\pm \sigma$, $\pm 2\sigma$ and $\pm 3\sigma$, and verticals have been erected

2	CLOSTRIDIUM WELCHII (CLOSTRIDIUM PERFRINGENS)	579
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3	CLOSTRIDIUM NOVYI (CLOSTRIDIUM OEDEMATIENS)	584
	MORPHOLOGY, 584, PHYSIOLOGY, 584, ANTIGENIC STRUCTURE AND TOXIN, 585, PATHOGENICITY FOR MAN, 585, PATHOGENICITY FOR ANIMALS, 586.	
4.	CLOSTRIDIUM HISTOLYTICUM	586
	MORPHOLOGY, 586, PHYSIOLOGY, 587, PATHOGENICITY, 587	
5	CLOSTRIDIUM SPOROGENES	587
	MORPHOLOGY, 588, PATHOGENICITY, 589.	
	<i>Blackleg (Clostridium Chauvei)</i>	589
	MORPHOLOGY, 589; PHYSIOLOGY, 590, PATHOGENICITY, 590, IMMUNIZATION, 591.	
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29	THE GLANDERS BACILLUS (MALLEOMYCES MALLEI)	599
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	<i>Malleomyces Whitmori (Malleomyces Pseudomallei)</i>	604
30.	CORYNEBACTERIUM (THE DIPHTHERIA BACILLUS)	606
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	<i>The Tubercle Bacilli</i>	625
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(0.5 + 0.5)", the curve with which we are actually concerned will usually be asymmetrical, corresponding, for example, to the expansion of (0.3 + 0.7)", for we shall not necessarily be dealing with a test that gives an average mortality of 50 per cent. But if the number of animals in the group under test is not too small, say not less than twenty, and the chance of the less likely event, life or death whichever it may be, is not less than 0.2, the odds calculated on the basis of the standard deviation are sufficiently accurate.

It will, however, have been noted that we have, throughout the preceding discussion, assumed the possession of one very important piece of knowledge—the true average mortality of the procedure under test. Usually we do not know this and have to assume that it is in fact the mortality we observe in the course of our experiments. When we compare two groups of animals, one treated, the other not, we may get a difference in mortality. This difference may have arisen by chance; i.e. the treatment has had no influence on the result. In this case the most probable event is a difference of 0. But the results from each group may by chance vary round the mean, and if one result happens to deviate largely in the positive direction and the other in the negative, the chance difference may be large. If we repeated the comparison many times, we should expect the larger chance differences to be infrequent, the smaller ones more frequent, and differences of 0 to be most frequent. Now it is found that *differences* between pairs of mean values derived from two normal distributions are themselves distributed normally; and this distribution of *differences* has a standard deviation, or as it is usually called, a standard error. This standard error is easily obtained, since it is the square root of the sum of the squares of the standard errors of the two means from which the difference was derived. In our simple binomial examples, where x_1 and x_2 are the numbers of animals dying in the two groups, the standard error of a mean proportional death-rate x_1/n_1 found in a group of n_1 animals in which the expectation of death is p_1 is $\sqrt{p_1 q_1/n_1}$, and similarly of x_2/n_2 is $\sqrt{p_2 q_2/n_2}$. The standard error of the difference $x_2/n_2 - x_1/n_1 = d$, is then

$$\sqrt{p_1 q_1/n_1 + p_2 q_2/n_2}.$$

If the difference has arisen by chance, i.e., if the two groups do not differ in any significant way, the best measure we have of the real chance of death is the death rate in the 2 groups as a whole. This, the mean chance of death, is $(x_1 + x_2)/(n_1 + n_2)$, which we may call p_0 . The mean chance of survival, $q_0 = (1 - p_0)$. The standard error, then, of the mean x_1/n_1 is now $\sqrt{p_0 q_0/n_1}$ and that of x_2/n_2 is $\sqrt{p_0 q_0/n_2}$; and the standard error of the difference is $\sqrt{p_0 q_0 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$ or if we are dealing in percentages

$$100 \sqrt{p_0 q_0 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

Let us now take a concrete example. Thirty mice that had been repeatedly inoculated with a killed suspension of *Salm. typhi-murium* and 30 uninoculated mice to serve as controls were injected intraperitoneally with a constant dose (1,000 bacilli) of living *Salm. typhi-murium*. They were then observed over a period of 28 days. During this time 16 of the 30 vaccinated mice died of the infection, and 29 of the 30 controls. We may tabulate these results as follows:

Vaccinated.	14 = 46.67 per cent.
Controls	1 = 3.33 " "
Difference	13 = 43.34 " "

What are the odds against each of these observed differences in behaviour having been due to chance?

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The experimental material can be tested for heterogeneity in various ways, though it is not often done. Lockhart (1926) divided a population of mice into groups of 20, gave each the same dose of a preparation of living *Salm. typhi-murium*, and observed them for 14 days. In one experiment with 9 groups, the deaths were 16, 16, 17, 19, 18, 17, 15, 17 and 14. This gives 36 differences between pairs. The largest observed difference, 19-14, should (Table 60) occur by chance only once in 27 comparisons. In a second experiment with 5 groups, the deaths were 18, 17, 13, 16 and 13. The largest difference, 18-13, occurs twice in 10 comparisons between pairs, and from Table 60, should occur by chance once in 17 comparisons. As far as they go, then, these figures and others we have not cited indicate within the limits of experimental error that Lockhart was reasonably successful in randomizing his experimental conditions. But had the distribution of deaths differed widely from that expected from the errors of random sampling, it would have been clear that disturbing factors had not been eliminated.

It is doubtful whether equally satisfactory results can always be attained in immunological tests of this kind. The possible disturbing factors are legion; so that unless there is good technical and statistical evidence of homogeneity, we shall be wise not to accept the usual odds of 20 to 1 as indicating a statistically significant difference in behaviour of two groups. We might, for instance, fix an arbitrary limit for the ratio of a difference to its standard error at 3:1, which would give us calculated odds of 369 to 1 against the observed difference being due to chance. These odds, may, of course, be too high, and might lead us to ignore differences that are possibly significant. But if we adopt lower odds we may attach significance to differences that are in fact meaningless. In dealing with observations from the ward, or from the field, where fresh results cannot quickly or easily be collected, it is desirable that attention should be drawn to any points of possible interest or importance, with due emphasis where necessary on the allowance that must be made for sampling errors. In experimental work the case is rather different. The investigator will clearly be wise to take any hint that is offered by his quantitative results; but if he desires to publish his findings and draw conclusions from them, it is reasonable to insist on a stricter standard. There is nothing to prevent his repeating any experiment that has given a statistically dubious answer, and this is clearly what he should do.

Experiments should, indeed, always be repeated, even when a single trial has given a result that passes all the conventional tests of statistical significance. We can never be quite sure that the conditions of a single trial do not include some determining factor other than that we are trying to investigate. By repeating an experiment several times, we are testing, in the only way we can, for the intrusion of unknown factors other than errors of random sampling. If each of several tests gives us a statistically significant answer, and each answer points in the same direction, we shall have some justification for stating our conclusion in general as opposed to particular terms; but if some tests give different answers from others we must solve the problem of why these differences occur before we can accept the indication given by the original experiment.

The question thus arises, assuming a limit to the number of experimental animals that we have available, how we can use them to the best advantage in solving any particular problem. Shall we carry out one, or two, experiments with large numbers of animals in our test and control groups? or, shall we make several experiments, each on a smaller scale?

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The answer would seem to be that each test should be made on a number of animals sufficiently large to enable us to detect a difference of the order we are expecting, making allowance for errors of random sampling. When this point has been reached we shall gain more by repeating the experiment, using groups of a similar size, than by doing one very large experiment, in which the effect of sampling errors would be considerably reduced, but which would give us no information in regard to other intrusive factors.

In most laboratory experiments, in which we are expecting a high mortality, usually approaching 100 per cent., in our control group, and are hoping for a much lower mortality, say 30-60 per cent., in our treated group, we shall gain more information by repeating our experiment six times, using 25 animals in each group, than by doing one experiment with 150 animals in each group. The best procedure, in any given case, will depend on the size of the difference we are expecting.

Sometimes, particularly in the application of a therapeutic measure in human disease, quite a small difference in mortality is an objective well worth attaining; and, if we wish to determine a difference of this size, our groups in any individual trial must be relatively large.

Let us suppose we have made an antiserum which, on *a priori* grounds, we expect to be of value in combating a certain infective disease. Like all new curative measures it *must* be subjected to trial before it is adopted for routine use. It is not a question of trial or no trial, but of a well-planned trial that will give us a definite answer as quickly as possible, as against a planless, haphazard series of trials that will take a long while to tell us very little, perhaps at a considerable expense in lives. Let us also suppose that the real average effect of the serum is to reduce the case fatality of the disease from 30 per cent. to 20 per cent.—not, perhaps, a dramatic result but one that will save a large number of patients if the disease is a prevalent one. The only way in which the existence of such an effect can be quickly and certainly established is to test the serum on an adequate group of cases and compare the mortality in this treated group with the mortality in an untreated group. We must ensure that the two groups are as alike as possible by making the comparison over the same period and in the same place, because the infectivity and severity of many diseases fluctuates with time and place; and we shall try, for example, to test only one race, and to have similar age and sex distributions in the two groups.

Table 61 sets out the figures for test groups ranging in size (n) from 10 to 500. In each case the 10 per cent. difference in mortality appears; but the standard error when $n = 10$ is 19 per cent., and though it decreases with increase of n (proportionately, in fact, to \sqrt{n}) it is not until $n = 150$ that the 10 per cent. difference is even twice the standard error. After a trial on two groups each of 200 patients we should begin to think it probable that the serum had done some good; and we might accept the decrease from 30 to 20 per cent. mortality as an approximate estimate of the benefit to be expected from the serum. That is, we save one patient in three of those who would otherwise have died. When trials on this scale were repeated we should be surprised if there were many unfavourable reports. After a trial on two groups of 500 patients we should have no reasonable doubt that our serum had reduced the case fatality.

It is of very real importance that trials of any new therapeutic measure should be planned and carried out on an adequate scale. Otherwise the period of so-called trial may be extended far beyond the necessary limits; and a useless measure

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days with a standard error of 0.960 days, whereas the mean time to death of the 28 mice was 5.93 days with a standard error of 0.924 days. The difference is 4.99 days, and the standard error of the difference is $\sqrt{0.960^2 + 0.924^2} = 1.33$. The ratio of the difference to its standard error is $4.99/1.33 = 3.75$, and the odds against observing such a difference as the result of chance (Table 59) are about 2,000 to 1. The conclusion is that we have almost certainly made the disease less acute as the result of immunization, although we have not increased the resistance of our mice to the point at which their chance of ultimate survival is significantly greater.

THE MEASUREMENT OF TOXICITY OR VIRULENCE

We often want to determine the minimal lethal dose of a bacterial toxin, or of a living bacterial culture. Still more often we want to compare the minimal lethal dose of two toxins, or of two cultures. Clearly we must first titrate graded doses of the two cultures against a number of groups of animals. The results of such titrations are instructive. When we divide a large number of similar animals into groups of equal size, and administer to each group one of a series of increasing doses of a lethal culture or toxin, we may observe, if the scale of doses was suitably chosen, that none of the animals dies in the group receiving the smallest dose. With each increase of dose a larger death rate is observed and in the group receiving the largest dose all the animals die.

TABLE 62

Dose <i>a</i> in Arbitrary Units	Percentage Mortality in Groups of 100 Animals	Increase in Percentage Mortality with each Increment of Dose
2	0	—
3	1	1
4	4	3
5	10	6
6	22	12
7	40	18
8	60	20
9	78	18
10	90	12
11	96	6
12	99	3
13	100	1

Table 62 shows a set of hypothetical percentage mortalities in 12 groups of 100 animals, each group being given one of a series of doses of a lethal toxin. Each dose differs by one unit of measure from the preceding dose. When we plot the death rate against dose, as in Fig. 241(A), we obtain a characteristic curve (Table 1927, 1929, 1930) for the toxin, or, more specifically, a dose-response curve. The third column of Table 62 records the values obtained by subtracting the mortality rate for a given dose of *a* units, the mortality for the preceding dose of (*a* - 1) units. Inspection both of column 3 and Fig. 241(A) reveals one of the most important features of the dose-response curve, namely that the rate of change of percentage mortality is greatest in the middle range of the curve. At the extremes, a relatively large change in dose results in a small change in mortality.

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of pathogenic bacteria, the dose-response curve is far less steep. The LD50 can be more accurately measured, and even with steep dose-response curves, it is to be preferred to an estimate of the 100 per cent. killing dose. For example, when a strain of *Salmonella typhi-murium* was given in graded doses, each into 25 mice, the rise in mortality with numbers of bacilli injected was as follows: 10 bacilli, 24 per cent.; 10^3 bacilli, 56 per cent.; 10^5 bacilli, 88 per cent., and 10^7 bacilli, 96 per cent. For 100-fold increases in dose the change in percentage mortality in the region of 95 per cent. was relatively small; it was greater in the region of the 50 per cent. killing dose. In essentials, then, toxicity or virulence is best measured in terms of the LD50.

It is instructive to inquire why the dose-response curve has this particular S-shape. If we assume that each group of 100 animals is representative of the total of 1,200 animals from which they were drawn, it is clear that the figures in column 3 of Table 62 represent the proportion of all the animals which are susceptible to a dose of a units, but not to a dose of $(a - 1)$ units. In each case, then, we may say that the recorded proportion of animals is on the average susceptible to a dose half-way between these two values, that is, $(a - 0.5)$ units. Thus, the 3 per cent. of the animals susceptible to 4 units but not to 3 units, are on the average susceptible to 3.5 units, and so forth. Plotting these proportions against doses as abscissæ we obtain the curve in Fig. 241 (C), which is in effect a frequency distribution of susceptibilities in the population of animals employed. The average, or mean resistance, corresponds to the LD50. The hypothetical curve B represents the response of another batch of animals to the toxin, estimated from a series of doses increasing by 0.4 unit. The curve (D), the distribution of susceptibilities derived from it, has been drawn on a scale of unit increases in dose, for comparison with the curve (C). It is clear that the steeper dose-response curve corresponds to a narrow distribution of susceptibilities obtained in the performance of the test. These distributions, it should be noted, are distinct from the distributions of chance effects discussed on p. 1120, which were derived from an ideal population of identical animals infected with a fixed dose. Here we are estimating how widely the population of animals varies in resistance to graded doses, though each mortality measurement is subject to the error discussed above. In estimating the mortalities from which the dose-response curve is built, we shall have to take into account both sources of error—the error due to heterogeneity of the animal population tested, and the error due to chance effects in small groups of animals. When the death-rate of a group is known, the last can be estimated by the \sqrt{npq} formula; the first, only by measuring the slope of the dose-response curve.

It will be clear that the more homogeneous the animals used, the more they approximate to an ideal test reagent and the smaller the error of measurement with a group of animals of a given size. Some breeds of laboratory animals used for biological assay of this kind are grossly heterogeneous, but it is possible to reduce the heterogeneity by inbreeding of selected strains. These inbred strains, which may or may not be pure lines, often yield much steeper dose-response curves than those of the parent strains, and their use in biological assay permits either a greater increase in the accuracy of an assay for the expenditure of the same number of animals, or measurements of the same accuracy with smaller numbers of animals. For example, Prigge (1937) recorded that the ratio of the smallest to the largest dose of diphtheria toxoid required to immunize the individual animals in a mixed laboratory strain of guinea-pigs against a standard injection of toxin was 1 : 32,000. Similar tests of pure-line strains inbred by brother-sister matings revealed a strain for which the ratio was as small as 1 : 25.

The LD50 may be estimated directly from a dose-response curve. One simple method is to fit a straight line to observed mortalities whose values lie between 25 per cent. and 75 per cent. and read off the LD50. Its accuracy depends on the assumption that the curve is approximately a straight line between these limits. It is limited in that

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TABLE 63

SHOWING THE NUMBER OF DEATHS IN 20 SAMPLES, EACH OF 12 MICE, INOCULATED WITH THE SAME SERIES OF GRADED DOSES OF THE SAME CULTURE OF PNEUMOCOCCUS.

Dose	Group.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
10 ⁶	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10 ⁵	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
10 ⁴	1	2	1	1	2	1	2	1	1	2	2	2	1	2	1	0	1	0	2	1
10 ³	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
10 ²	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

100 times more virulent than strain 18; whereas, as this experiment showed, there would have been no adequate reason to assume any difference in virulence.

THE MEASUREMENT OF IMMUNIZING POTENCY

We have already emphasized (p 1125) that the species of animal and of bacterium we use for immunological tests vary not only from day to day within a given laboratory, but from laboratory to laboratory. It follows that, strictly speaking, many of the results of a test are valid only for the particular biological system—such as mice and *Str. pneumoniae*—used on that occasion. By using mice of a known and obtainable breed and keeping them on defined diets, etc., and by using a known strain of pneumococcus whose constant virulence is assumed, say, by animal passage or preservation in the freeze-dried state, we may attempt to specify the conditions under which we, or other investigators, may expect to repeat our results. But the variability and heterogeneity of biological systems as complex as whole animals reacting with a living pathogen are quite beyond exact specification, and all we can hope to achieve is a broad similarity of circumstances, from the exploitation of which we shall arrive at approximately the same conclusion as before. When, however, such tests are made in order to specify an immunological product like a vaccine or an antitoxin for human use, the range of unpredictable variation within biological test systems may be so large that the answer we get from it is clinically unreliable. Suppose we are testing the immunizing potency of diphtheria toxoid on an apparently average batch of guinea-pigs. If in fact the animals were exceptionally easy to immunize, we should overestimate the potency of a given weight of the toxoid; and we should underestimate it with guinea-pigs of more than average resistance to immunization. Diphtheria toxoid happens to be relatively harmless in large doses, so that in this instance we can allow for overestimation of potency by immunizing with plenty of toxoid. This will mean that very large amounts of an underestimated toxoid may be unwittingly injected, which is wasteful, but not dangerous. With more toxic substances, obviously we cannot risk making such allowances for overestimated potency. Moreover, the allowances we make are in all cases largely guess-work. For these reasons this method of direct estimation of potency from the magnitude of the response in the test animal is to be avoided whenever possible. Fortunately we can often eliminate some of the uncertainty about the reproducibility of the biological test system by introducing standard preparations of the active substance

THE HISTORY AND DEVELOPMENT OF BACTERIOLOGY¹

Bacteriology illustrates, perhaps better than any other branch of biology, the complex system of interrelationships among the sciences. Since the microorganisms are so small that they are invisible not only to the naked eye but to the eye aided by simple lenses, their very discovery necessarily depended upon the development of optical science to such a point that lenses of sufficient perfection and magnifying powers became available. It is not strange, therefore, that the many evidences of bacterial activity, such as the putrefaction and decay of organic matter, the infectious diseases of man and lower animals, the common fermentations and similar natural phenomena, should have been viewed in the past as semimetaphysical processes. Despite the entire lack of evidence, some of the older writers voiced a belief in the existence of such microorganisms. Fracastorius of Verona (1546) suggested a *contagium vivum* as a cause of disease, and von Plenciz (1762) accounted for the specificity of disease on the basis of microbic etiology. Although such speculations might be considered a result of prophetic insight, in all probability they were no more than guesses to which, in view of present knowledge, it is not difficult to ascribe significance. In the absence of the experimental approach, the fertility of philosophic speculation remains a doubtful quality.

Kircher had reported the observation of "minute worms" in the blood of plague patients in 1659, but it is doubtful that he ever saw the plague bacilli. The first concrete foundations of bacteriology were laid by a Dutchman, Antonj van Leeuwenhoek, in the latter part of the seventeenth and the early part of the eighteenth centuries. Leeuwenhoek, a man of indefatigable industry and great curiosity, held a political sinecure in his native town of Delft. In his odd time, of which he apparently had a good deal, he ground lenses and became the most skilful lens grinder of his time. He utilized these lenses in the construction of simple microscopes and spent many years of his long life (1632-1723) in examining a great variety of natural objects, with unremitting industry if without system, and in the course of his observations chanced to come across the organisms now known as bacteria. That he did in fact observe these creatures is evident from the drawings he left of them. In a communication to the Royal Society,² of which he was a member, he states,

¹ For details see Bulloch: *The History of Bacteriology*. Oxford University Press, New York, 1938.

² Phil. Trans. Roy. Soc., 1677, 11-12 821. Observations, communicated to the publisher by Mr. Antony van Leeuwenhoek, in a Dutch letter of the 9th of Octob. 1676, Here English'd Concerning little animals observed by him in rain- well sea and snow water, as also in water wherein pepper had lain infused

obtained by comparing doses of serum that protected mice against the same challenge dose of *Salm. typhi* was 2 : 1 ; but by the less valid, but nevertheless often used, method of comparing the challenge doses of bacteria required to produce the same mortality rate in mice protected by the same volume of the two sera, the potency ratio was 29 : 1.

We can express the ratio (potency of "unknown" : potency of standard) directly, saying, for example, that, weight for weight, the unknown is 0.03, or 40.0, times the activity of the standard. It is more usual to assign unit potency

TABLE 64

SHOWING THE IMMUNOLOGICAL SUBSTANCES RECOGNIZED INTERNATIONALLY AS BIOLOGICAL STANDARDS OR REFERENCE PREPARATIONS.

International Standards.	Weight of the Preparations with Unit Potency.
Diphtheria antitoxin	0.0028 mgm.
Diphtheria toxoid, plain	(No unit defined)
Tetanus antitoxin	0.3094 mgm.
Tetanus toxoid	0.0300 mgm.
Gas gangrene antitoxin (<i>perfringens</i>)	0.1135 mgm.
Gas gangrene antitoxin (<i>oedematiens</i>)	0.1135 mgm.
Gas gangrene antitoxin (<i>histolyticum</i>)	0.2000 mgm.
Gas gangrene antitoxin (<i>Vibrio septique</i>)	0.0974 mgm.
Gas gangrene antitoxin (<i>Sordelli</i>)	0.1334 mgm.
Staphylococcus alpha antitoxin	0.2376 mgm.
Staphylococcus beta antitoxin	2.6230 mgm.
Anti-dysentery serum (<i>Shiga</i>)	0.0500 mgm.
Anti-pneumococcus serum (Type I)	0.0886 mgm.
Anti-pneumococcus serum (Type II)	0.0894 mgm.
Anti-A blood grouping sera	0.3465 mgm.
Anti-B blood grouping sera	0.3520 mgm.
Old Tuberculin	0.0100 mgm.
Mammalian P.P.D. (purified protein derivative)	0.000028 mgm.
Anti-typhoid serum	No units defined
Scarlet fever (<i>streptococcus</i>) antitoxin	0.049 mgm.
Anti- <i>Brucella abortus</i> antiserum	0.091 mgm.
Anti-Q Fever serum	(No unit defined)
International Reference Preparations.	
Cholera Agglutinating serum (Ogawa)	No units defined
Cholera Agglutinating serum (Inaba)	
Cholera Antigen (Ogawa)	
Cholera Antigen (Inaba)	
Cholera Vaccine (Ogawa)	
Cholera Vaccine (Inaba)	

(For immunological standards in preparation, see Report 1954.)

to a certain weight or volume of the standard, and express potencies as so many units per ml. or per gm. Thus the solution of the international standard for diphtheria antitoxin, as issued for use, contains 10 units per ml. If the ratio of equipotent volumes of standard and unknown proves to be 0.025, then the unknown contains $10/0.025 = 400$ units per ml.

The refinements of potency comparisons that lead to results exemplified in Fig. 242 are not always practised in immunological work. As we noted on page 1131, the dose response to exotoxins is steep, so that the interval between the maximum ineffective dose and the minimum fully effective dose, even when

"Having several times endeavoured to discover the cause of the pungency of pepper upon our tongue, and the rather, because it hath been found, that though pepper had layn a whole year in vinegar, yet it retained still its pungency, I did put about $\frac{1}{3}$ of an ounce of whole pepper in water, placing it in my study, with this design, that the pepper thereby being rendered soft, I might be enabled the better to observe what I proposed to myself. This pepper having lain about three weeks in the water, to which I had added some snow water, the other water being in great part exhaled, I looked upon it the 24th of April, 1676 and discern'd in it, to my great wonder, an incredible number of little animals, of divers kinds, . . . The 4th sort of creatures, which moved through the three former sorts, were incredibly small and so small in my eye, that I judge, that if 100 of them lay one by another, they would not equal the length of a grain of coarse sand; and according to this estimate, ten hundred thousand of them could not equal the dimensions of a grain of such sand." Leeuwenhoek is known to have used only simple lenses, and the highest magnification he reached was approximately 300 diameters. Probably he did not observe objects as small as bacteria by transmitted light. He kept his method of illumination secret; it is not improbable that he used reflected light, similar to that used in present day ultramicroscopes. Although his observations did not lead to an immediate development of bacteriology, their significance was apparent to many of the scientific men of the day. Slare, in a comment on a report³ on an epidemic among cattle, concludes with the remark, "I wish Mr. Leeuwenhoeck had been present at some of the dissections of these infected Animals, I am perswaded he would have discovered some strange Insect or other in them."

Nearly a century later, in 1786, the Danish zoologist, O. F. Muller, studied these microorganisms and succeeded in discovering many structural details of which his predecessors had been ignorant. He depicted several kinds of bacteria so accurately that they can be identified today as belonging to one or another of the chief divisions.

Another unequivocal advance was made by Ehrenberg (1795-1876). His principal work upon the "infusion animals" or "Infusionstierchen," as these organisms were then termed, was published in 1838 and brought together much more definite and detailed information concerning bacteria than had been previously secured. The chief merit of Ehrenberg's work lay in the system that it introduced into the study of microorganisms. He was able to establish a number of different groups among the organisms now known as bacteria, and recognized clearly the fundamental differences between the larger forms, such as the screw-shaped or spirally-twisted organisms, and certain of the true protozoa with which they had heretofore been classed. Some of the names which Ehrenberg conferred upon his "infusion animals," such as bacterium and spirillum, are still current in bacteriological nomenclature, although with changed signification.

³ Phil. Trans. Roy. Soc., 1682, 13 93. An abstract of a letter from Dr. Wincler, chief physician of the Prince Palatine, Dat. Dec. 22 1682 to Dr. Fred Slare, Fellow of the Royal Society, containing an account of a Murren in Switzerland, and the method of its cure. A further confirmation of the above mentioned Contagion, of its nature, and manner of spreading by way of Postscript from the ingenious Fred Slare, M. D. and F. R. S., Dat. March 27, 1683

should lead us to overestimate the support that a statistically significant result can give to the particular hypothesis the experiment was intended to test. As Fisher (1950a) points out, all that a properly designed experiment can test is the "null hypothesis"; that is, the hypothesis that there is no difference between the control and test systems that we are studying. If, for example, the calculated odds prove to be 3 to 1 against a chance effect in the defined conditions of a therapeutic test of a fluid A in a fatal bacterial infection, *no one could decide, without further experiment, whether the effect observed was due to "chance" or to A.* If the odds prove to be 1,000 to 1, we can say that the effect is unlikely to be an example of a chance variation of the kind we expect from the materials used in the test; but we cannot say that it was *therefore* due to the administration of a few millilitres of the fluid A. The statistically significant result indicates that the test and control groups differ in some important respect and therefore permits us to consider the fluid A as a possible cause of that difference; but the decision that A is the cause of the observed reduction in mortality in the test group is independent of any statistical demonstration of significance, and in a final analysis depends on the experimenter's knowledge of his particular science and his appreciation of the consistency of his ideas of A's action with the general theory of that science. If A was in fact water, the conclusion, no matter how statistically significant the experimental result, that a small quantity of water had cured the infection, would rightly be considered as suspect. If A, on the other hand, was a solution of a substance known to be antibacterial and non-toxic to the animals used in the experiment, then these pieces of scientific knowledge, coupled with the disproof of the null hypothesis, would go far towards justifying the conclusion that A was capable of curing a particular type of infection.

The aim, then, of the statistical method in experiments involving the comparison of two or more groups of events is to discover the probability that the null hypothesis is untrue; and only in those cases where the null hypothesis is disproved is there any justification for considering the possibility that an observed effect resulted from any of the deliberate variations in the conditions of the experiment.

The proper design and analysis of quantitative tests is essential for the efficient practice of a science. That the record of immunological work in the past contains few examples of statistical analysis is no proof that such analysis is unnecessary. Sometimes the results of an experiment are so clear cut that any arithmetical analysis is unnecessary; on many other occasions it will be found that the data are incapable of supporting the conclusions reached by the investigators. There is no doubt that much of value has been learned without any consideration of statistical design, but it is justifiable to suppose that the knowledge could often have been achieved with a great deal less expenditure of time and materials had the experiments been designed more efficiently.

The voyager who ignores tidal streams and compass deviations will arrive somewhere in the long run; but he is less likely to identify correctly the place at which he has arrived than is another who allows for these variables by the accepted methods of elementary navigation.

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In the two or three decades succeeding Ehrenberg's work, considerable knowledge was amassed concerning the mode of development and physiology of bacteria, as well as their position in biological classification, but the labors of Dujardin, Perty, Cohn, Nageli and others, although important, are quite overshadowed by the work of Louis Pasteur (1822-1895).

Up to the period of Pasteur's investigations, the role played by bacteria in various familiar natural processes, such as putrefaction, decay and fermentation, had been, perhaps, vaguely suspected, but had not received conclusive demonstration. Pasteur, originally trained as a chemist, had done his early work on stereoisomerism. The formation of optically active amyl alcohol during the course of the lactic acid fermentation attracted him to the study of the

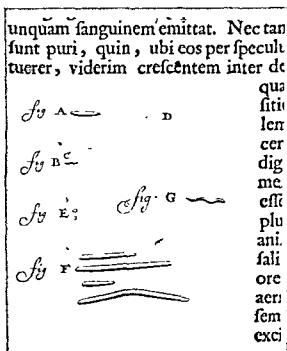


Fig. 1. The first pictorial representation of bacteria. Reproduced from *Arcana Naturae delecta ab Antonio van Leeuwenhoek*. Delphis Batavorum apud Henricum Crooneveld. 1695.

fermentation processes. The demonstration of the plant-like nature of yeast by Caignard-Latour and by Schwann, together with the asymmetric synthesis of amyl alcohol, led him to suspect that fermentation was a result of the activity of living cells. This view was strongly opposed by the chemists of the day, Liebig, Berzelius and Wohler in particular, who regarded the presence of dead and dying yeast cells of importance only in that in the course of their own molecular disintegration they toppled over and dragged down certain complex organic molecules with which they were in contact. Pasteur's researches led him deeper into the morass of fundamental biology than, perhaps, he had anticipated, for, before the essentially biological basis of fermentation could be conclusively demonstrated, the problem of spontaneous generation of life from decomposing organic materials presented itself for solution.

CHAPTER 44

THE MECHANISMS OF BACTERIAL INFECTION

BEFORE we can discuss the mechanisms by which the host resists infection, we need as clear a picture as possible of the ways in which the parasite injures the host. Some parts of the picture are confused, others sketchy; but parts of the main outline are quite clear and definite, and it is with these that we shall be mainly concerned.

Specific Infection. Koch's Postulates.—

Although not immediately pertinent to our main problem, a brief discussion of the kind of evidence which will justify the conclusion that a particular disease is caused by a particular parasite forms a convenient introduction to infective processes in general, and raises points of some importance.

A number of conditions which must be fulfilled in order to establish such a causal relationship are set out in most bacteriological textbooks under the title of "Koch's Postulates," though Koch (1891) did not enunciate them in the categorical form in which they are usually quoted. They run in general terms as follows:

- (1) The organism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.
- (2) The organism should be cultivated outside the body of the host, in pure culture, for several generations.
- (3) The organism so isolated should reproduce the disease in other susceptible animals.

To-day we recognize additional criteria of a causal relation between a micro-organism and a disease, of which one of the most valuable is the demonstration of specific antibodies in abnormally large amounts in the blood of an infected man or animal, or an abnormally high degree of specific immunity to the infecting agent in a recently recovered animal.

It is obvious that technical difficulties may prevent us from fulfilling all Koch's postulates in every disease. We may be unable to cultivate the causative parasite although we can demonstrate its constant presence in the lesions by suitable staining methods; this has hitherto been the case with regard to the bacillus of leprosy. The organism may be so small, and its morphology so doubtful, that we are unable to demonstrate its presence in the tissues, or to distinguish it with certainty from other bodies of the same order of size. When we cannot cultivate the organism *in vitro*, we may, as in the initial studies on most virus infections, have to rely on the third postulate alone, and reproduce the disease in a suitable animal by the inoculation of filtrates from infected blood or tissue extracts that contain no visible or cultivable bacteria. In other cases the third postulate may be our stumbling-block. We may be able to relate a characteristic bacterium to the

For a great many years it had been generally held that living things, even organisms as large and complex as mice, were spontaneously generated during the course of the decomposition of organic substances. The experiments of the poet physician Redi (1626-1697), however, indicated definitely that maggots were not spontaneously formed from decomposing meat but were in fact fly larvae hatched from eggs deposited in the meat by flies. Spallanzani, an Italian monk, showed further that putrescible meat infusions, when properly heated, did not spoil and did not contain living organisms even though kept over long periods of time. Needham, an Irish priest, took issue with Spallanzani on the basis of similar experiments in which spoilage took place and living organisms appeared in spite of previous heating. A second series of elaborate experiments by Spallanzani corroborated his earlier findings and indicated the fallacies in Needham's experiments. Other experiments by Schulze, Schwann, Schroder and von Dusch indicated that no spontaneous generation took place but rather that the source of living organisms was the air in which they were suspended, a conclusion amply confirmed by the careful experiments of the Englishman Tyndall. The entire controversy was revived by the extended experimental work of Pouchet, which appeared to indicate a spontaneous generation of microorganisms in heated organic materials; and Pouchet created a great stir in French scientific circles—a stir in which Pasteur found himself.

The point at issue was, obviously, whether spoilage of such infusions took place as a result of the presence of microorganisms or whether the appearance of living organisms was a result of the decomposition—a general question of which the question of the biological basis of fermentation was a special case. Pasteur's extended and careful investigations showed beyond reasonable doubt that microorganisms were present in air, the numbers varying with the place from which the air came; that putrescible material which had been heated sufficiently to destroy all life would keep indefinitely with no evidences of either decomposition or the presence of living organisms, and, finally, that a common source of contamination of such sterilized material was air in which living microorganisms were suspended.

The fact that life came only from life (biogenesis) and was not spontaneously generated from non-living materials (abiogenesis) having been established,⁴ it was not difficult to prove that fermentations resulted from the physiological activities of living, growing microorganisms. Furthermore, the specificity of fermentations, the fact that different kinds of fermentations were consequences of the activities of different kinds of microorganisms, grew out of this work. The transition from studies on fermentation to studies on infectious disease was not a difficult one to make, for there was a growing awareness among scientific men of the day of the similarities between the development of disease in an individual and the fermentation of sugar solutions.

Pasteur was led into a study of the "diseases" of beer and wine, processes which he found to be none other than secondary fermentations brought about through the activities of extraneous microorganisms, decompositions that resulted in the accumulation of end products of an undesirable nature. He

⁴ Speaking scientifically. Philosophic speculation is beyond the scope of the present volume.

There are two distinct stages in the use of experimental animals in the study of an infective disease. In the first, as demanded by Koch's third postulate, we are trying to reproduce a particular infection in a form which is clinically or pathologically recognizable as identical with the natural disease. In the second we are using our susceptible animal for an intensive study of this infective process. The lesions produced in the guinea-pig by the subcutaneous inoculation of cultures or filtrates of the diphtheria bacillus bear little resemblance to diphtheria as it appears in man; but almost the whole of our present knowledge of immunity in relation to that disease, which has at last placed us in a position to exert effective control, has been built up on knowledge gained from experiments on that small laboratory animal.

It should be noted, also, that there is a shifting of emphasis from the clinical to the bacteriological side as our knowledge progresses. At the start of our inquiry we are presented with a clinical or epidemiological phenomenon, and our task is to discover the microbial parasite that is the necessary cause. We may, as in the case of diphtheria, or syphilis, or tetanus, find that a single specific parasite is responsible; indeed this is far the most frequent solution if we neglect racial variations within the causative microbial species. We may, however, find that a disease characterized by one clinical syndrome may be caused by one of several distinct species of bacteria. Secondary pneumonia affords one example, bacillary dysentery another. Once the connection between a particular parasite and a particular disease has been firmly established, the tendency of the bacteriologist is to focus his attention on the organism, rather than on the clinical condition to which it most frequently gives rise, and to try to build up a picture of the reactions between that organism and a susceptible host species in general. Thus we should think in terms of pneumococcal infection, rather than in terms of pneumonia; and regard that particular condition merely as one example of the reaction between the pneumococcus and the human tissues, including in our generalized picture such lesions as middle-ear disease, sinusitis, meningitis, peritonitis, suppurative arthritis, etc., and keeping constantly in mind the part played by bacteræmic spread and selective localization.

It will be convenient, in this and the immediately succeeding chapters, to deal exclusively with bacterial infections, since most of our knowledge has been acquired in studying infections of this type. In a later chapter we shall discuss how far our conceptions have to be modified in the case of infections with the filtrable viruses. Protozoal and helminthic infections are beyond the scope of this book; though the methods elaborated by the bacteriologist are proving fruitful in their application to this wider field.

Invasion of the Tissues by Bacteria.

With very few exceptions, bacteria and other microbial parasites must gain access to the host's tissues and multiply in them before they can produce any harmful effect; and we may for the moment regard the capacity to pass through some protective covering layer—most commonly through one of the mucous surfaces of the body—and to multiply in the subjacent tissues, as an essential attribute of a pathogenic organism. The "passing through" must not, of course, be interpreted too literally, or as necessarily implying any active motion on the part of the parasite. Apart from gross mechanical injuries to the skin and mucous membranes, the routes by which micro-organisms and inert particles of non-living matter get into

controlled the fermentative process by gentle heating followed by inoculation of the fermentable mixture with microorganisms which brought about the desired type of decomposition. The heating process has been termed "pasteurization" in his honor. A further development was a study undertaken at the request of the silk growers, of pébrine, a disease of silk worms which was assuming considerable economic importance. Here too a microbic etiology was discovered and practical control measures could be and were applied.

The so-called germ theory of disease had, at this time, permeated more and more generally into the scientific thought of the day, largely, perhaps, as a result of the strong support it received from Pasteur's researches on fermentation. Lister (1827-1912), an English surgeon, was one of the first to grasp the significance of Pasteur's work in relation to human disease, and put the new concepts into practical operation with his introduction of the antiseptic technique into surgical operations. The free use of strong carbolic acid brought about a marked decline in surgical mortality, which had been terrifically high in even the most minor operations. It remained, however, for Robert Koch (1843-1910), a German physician, to develop the experimental methods necessary to the proof of the causal relation between bacteria and infectious disease.

One of the greatest difficulties encountered by the early workers in bacteriology was that of separating bacteria from one another. That morphologically different types existed was clear, and, further, that organisms exhibiting the same morphology differed from one another in ability to produce disease had been apparent from the early work on pyemia. Perhaps one of the greatest single contributions to bacteriology is the method of isolation of bacteria in pure culture developed by Koch. It consisted, essentially, of culture on a semisolid medium, a nutrient environment solidified by the addition of gelatin or agar-agar—a method so simple and yet so effective that it is used practically unchanged today (p. 20). Koch had achieved what he had said only a year or two before was "impossible."

Although it was almost entirely through the works of Pasteur that bacteria and other microorganisms emerged from their relative obscurity as organisms of interest chiefly to the professional biologist and took a conspicuous position in natural science as a group of organisms whose activities and capabilities were full of a far-reaching significance for all mankind, the contributions of Koch and the German school were also of fundamental significance. If any one man can be looked upon as the founder of bacteriology, that man is Louis Pasteur. Likewise, the infant science owed its firm experimental foundation directly to Koch, a foundation without which it could never have become a science.

The isolation of bacteria in pure culture, coupled with the methods of staining developed by Koch, Ehrlich, Wiegert and others, so markedly stimulated the study of bacteriology that the ensuing decade or two, the eighties and nineties, became in truth the first golden age of that science. As the history of all science is, in essence, the history of its methodology, so in bacteriology discovery of the specific etiology of a variety of infectious diseases followed rapidly upon Koch's studies of anthrax and his isolation of the anthrax bacillus. Koch himself isolated and described the tubercle bacillus in 1882 and the cholera

the parasite and the resistance of the host. A highly virulent strain may in a resistant host produce a disease indistinguishable from that due to a strain of low virulence in a susceptible host. When we talk of virulence, either the host and its condition is specified, or we imply "virulent" in a species of host of average resistance. If we do not, any comparisons of virulence are meaningless. The same is true of statements about host resistance: a train of parasite is specified, or a parasite of average virulence is implied.

It may, then, be stated that multiplication of bacterial cells within the tissues is one of the essential factors on which infection depends. Some species can produce a fatal infection without any wide dissemination within the body: others cause death only when such widespread dissemination occurs. A part of the mechanism of resistance will clearly be concerned with the killing and removal of the bacterial cells as such.

Bacterial Localization.

Apart from the generalized bacteremic invasion referred to above, most bacteria tend to lodge and multiply in tissues remote from their primary lodgment; and certain species, or groups of species, differ sharply from one another in the site of such localization.

The site of the primary lodgment and the direction of the initial spread of infection are largely determined by the portal of entry to the body, and by the local anatomy, particularly by the direction of the lymph flow. But the portal of entry in its turn is determined by the natural habitat of the parasite, and this by its potentialities for life under different environmental conditions.

Thus, some species of staphylococci are normal inhabitants of the skin. Those species, or races, that are potentially pathogenic, may gain access to the underlying tissues by the hair follicles, or by the ducts of the sebaceous glands, and cause the characteristic pustules or boils.

Many of the bacteria that lead a parasitic existence in the nasopharynx, and are spread from host to host in droplets, droplet-nuclei and dust, tend in consequence of a common respiratory portal of entry to show a rough similarity in the distribution of the lesions they produce; but the similarity is very incomplete, and breaks down altogether on a close analysis. Thus, pneumococci, hæmolytic streptococci, influenza bacilli, diphtheria bacilli and meningococci are all spread in this manner. Diphtheria bacilli and hæmolytic streptococci both tend to lodge and multiply in the tonsils, the former giving rise to the characteristic lesions of faucial diphtheria, the latter to tonsillitis. Both produce soluble toxins, which diffuse widely from the initial lesion. But the tendency to invasive spread is much greater in the case of the streptococcus than in that of the diphtheria bacillus. Pneumococci, hæmolytic streptococci and influenza bacilli may all spread to the lungs and cause pneumonic lesions, but they do it in very different ways. The pneumococcus fully merits its name by its pre-eminence as an invader of the pulmonary tissue. It is the sole important bacterial agent in the primary pneumonia of adolescence and adult life. In the secondary pneumonias associated with such infections as measles, whooping cough and influenza, it shares its honours with the influenza bacillus and the hæmolytic streptococcus. The meningococcus, although it is a nasopharyngeal parasite and spreads by droplet infection, produces its characteristic lesions in the meninges. Pneumococci, hæmolytic streptococci and influenza bacilli may also give rise to meningitis; but whereas this is the modal reaction of the meningococcus when it produces clinically obvious infection, it is

vibrio the following year. Other discoveries tumbled in one after another with bewildering rapidity. Klebs described the diphtheria bacillus in 1883, and in 1884 Löffler isolated and studied this organism; Fraenkel discovered the pneumococcus in 1886, and the following year Weichselbaum isolated the meningococcus; Kitasato cultivated the tetanus bacillus in 1889, and in 1894 he and Yersin discovered the plague bacillus independently. These and many other similar discoveries made clear to the world in a striking fashion the significant implications of the new science.

Meanwhile, the investigations of the French workers had taken a somewhat different turn. Many years before, in 1796, Jenner, an English physician, observed that infection of human beings with cowpox, a disease of cattle closely resembling smallpox, protected against subsequent infection with smallpox. Pasteur, investigating first chicken cholera and later anthrax and swine erysipelas, succeeded in demonstrating a basic principle of immunology, that inoculation with attenuated microorganisms, those which by some sort of treatment had lost their virulence, resulted in the development of an increased resistance or immunity of the inoculated individual against later infection with the same organism. Pasteur's most striking application of this principle was in the development of a prophylactic treatment against rabies—an accomplishment with some elements of the dramatic because of the dread in which the disease was held. Not long afterward the American workers, Salmon and Theobald Smith, discovered that the inoculation of killed bacteria would also stimulate the development of the immune state. The cellular theory of immunity propounded by the Russian zoologist, Metchnikoff, was soon overshadowed by the discovery of tetanus antitoxin by von Behring and Kitasato and, shortly afterward, the discovery of diphtheria antitoxin by von Behring, and the precise studies of the humoral antibodies by Bordet and others; only in recent years has it come into its own. The utilization of the immune phenomena in the diagnosis of disease by Wassermann, Widal and others, Paul Ehrlich's development of his side-chain theory of immunity and his initiation of chemotherapy through the synthesis of salvarsan all contributed to the expansion and development of bacteriology. The discovery of the filterable viruses, apparently living agents too small to be seen with the most powerful microscopes, by Iwanowski, Beijerinck, and Löffler and Frosch brought up new problems, many of which are still unsolved today.

The new science, sired by Pasteur and nurtured by Koch, expanded beyond medicine and infectious disease into agriculture and the industrial fields. The genius of the Russian worker, Winogradsky, made possible the elucidation of the perplexing problems of soil fertility by his isolation of autotrophic bacteria which oxidized ammonia to nitrites and nitrates. The discovery of the nitrogen-fixing bacteria living symbiotically with leguminous plants by Hellriegel and Wilfarth and of the free-living nitrogen-fixing forms by Winogradsky and by Beijerinck further clarified the puzzles of soil fertility and indicated the important functions of the soil bacteria. The discovery of the bacterial etiology of pear blight by Burrill and the isolation of bacteria responsible for many other diseases of plants have shed new light on old agricultural problems. It has also been discovered that other kinds of bacteria impart the characteristic flavors or aromas to butter, cheese and other dairy products; and that still

competent histologist could distinguish them without bacteriological assistance. The special case of tuberculosis is of particular interest, in that a beginning has been made in tracing the connection between the various chemical products of the bacterial cell and the type of tissue reactions to which they give rise (see p. 496). Another example is the non-toxic, non-antigenic lipid isolation from *Erysipelothrix monocytogenes* by Stanley (1919), which induces in rabbits the monocytosis that characterizes the natural infection by this organism.

It is not, of course, true that every pathogenic bacterium provokes a distinct and characteristic cellular response, or that the pathologist unaided can often arrive at an exact bacteriological diagnosis from a histological examination of the infected tissue; but, as Kettle (1927) has pointed out so clearly, there is a broad correlation between certain types of lesion and certain groups of bacteria; and the groups which the pathologist would recognize on the basis of the distribution and type of the lesions produced are, in many cases at least, those which the bacteriologist would recognize as containing closely related bacterial species.

All this is perhaps obvious and elementary; but it is of the first importance to realize that different pathogenic parasites have different points of attack, and different methods of attacking.

Bacterial Characters Associated with Pathogenicity.

There is no apparent reason why the mere presence in the tissues, or blood stream, of any reasonable number of cells of an order of size expressed in low multiples of μ should exert any harmful effect on the host that harbours them. No mechanical theory of the pathogenic action of bacteria is compatible with our knowledge of the way in which the tissues deal with inert particles which have gained access to them. The basis of all harmful effects of bacterial infection is quite certainly chemical; and only when the chemist has replaced the immunologist shall we be able to give an intellectually satisfying account of what happens when a particular parasite invades a particular host. In the meantime we must do what we can with the crude data at our disposal.

It has long been known that certain bacterial species produce highly poisonous substances which give rise to characteristic lesions or symptoms when injected into susceptible animals. These are the bacterial toxins. Besides these obvious toxins, less toxic substances are found in the bacterial bodies, and among the soluble products of bacterial growth, which act on tissues or tissue constituents and which may directly intoxicate the infected host, or play an auxiliary part in promoting invasion.

It is fairly easy to establish the pathogenic rôle of a frank bacterial toxin, especially when it is antigenic. Taking the toxin of *Cl. tetani* as an example, we find that: (a) it is produced by the tetanus bacillus during an infection, because the body responds by producing specific antibody; (b) virulence and toxigenicity are associated since virulent strains of *Cl. tetani* produce the toxin, whereas avirulent strains do not; (c) injection of toxin alone mimics the natural disease; (d) active immunization with toxin protects against tetanus; and (e) antitoxin passively immunizes against tetanus.

When the substance produces no characteristic intoxication, or is not toxic although suspected of having an auxiliary rôle in infection, evidence of the kind in (a) and (b) is not conclusive, because the body responds to all bacterial antigens, irrespective of their pathogenic rôle; and the association of a substance with the

others determine the success or failure of various industrial processes, such as the retting of flax, the tanning of hides and, perhaps, the curing of tobacco.

Thus, in the space of only a few decades, bacteriology had become a full-blown, vigorous young science, fully capable of standing on its own feet—and this from Leeuwenhoek's "little animals" that behaved in such curious ways.

The outstanding success of the practical applications of bacteriology should not be allowed to overshadow the fact that it owes its present important place among the biological sciences quite as much to its general scientific significance. It has been often pointed out that bacteriology has produced a change in man's conceptions of the world around him so sweeping as almost to deserve the term "revolutionary." Up to the middle of the nineteenth century the character of many of the most familiar natural processes, such as decay, fermentation and the like, was entirely misunderstood, contemporary spontaneous generation of at least the lower forms of life was the generally accepted belief of most scientific men, infectious diseases were not sharply differentiated from one another and the most fantastic hypotheses were advanced to explain their existence. Although the great mass of material phenomena elsewhere had been brought into apparent orderliness and system, here was a region in which the unscientific imagination rioted in mystery and extravagance. The penetration of this realm of obscurity by the discoveries of bacteriology gave the human race for the first time in its history a rational theory of disease, dispelled the myths of spontaneous generation, and set the process of decay and kindred phenomena in their true relation to the great cycle of living and non-living matter. The new conception of the microscopical underworld which bacteriology brought into biological science must be reckoned as a conspicuous landmark, and, in so far as it has changed the attitude of man toward the universe, should be regarded as one of the most important triumphs of natural science.

Underlying all the applications of bacteriology are certain fundamental facts and principles concerning the structure, mode of development, and general physiologic requirements and capabilities of bacteria themselves. This subject matter constitutes the ground work of bacteriology, and is essential not only to a proper comprehension of the present practical applications of bacteriology, but also to the further development of the science.

endotoxin is of the order of 5-25 mgm. for mice—an enormous dose compared with the corresponding figure (see above) of 0.0016 μ gm. for botulinum toxin. They are relatively stable, withstanding temperatures of 100° C. for over 1 hour.

Finally, endotoxins are seldom strongly antitoxinogenic, though they may stimulate a good agglutinin, precipitin or protective antibody response. With some organisms it has proved impossible to prepare anti-endotoxic sera. More commonly prolonged immunization of an animal yields an antiserum which is able to neutralize a few M.L.D. of the endotoxin; but the simple linear relationship between the amount of toxin and antitoxin in a just neutralized mixture that obtains with exotoxins does not hold with endotoxins. Often no amount of increase in antitoxin affords protection when the dose of endotoxin passes a certain limit.

Although this broad distinction can be made by comparing typical exotoxins with typical endotoxins, not all toxins fall simply into two well-defined groups. Thus, the toxin of *Cl. botulinum* is relatively heat-resistant (75°-80° C. for 10 min.); so is the exotoxin of *Sh. dysenteriae* (Anderson *et al.* 1945). The α -toxin of *Cl. welchii* has a relatively large L.D 50, and is relatively heat-resistant (70° C. for 30 min.). The erythema-producing toxin of the hæmolytic streptococcus—the toxin which is responsible for the rash in scarlet fever, and is employed in the Dick reaction—is filtrable, has a characteristic action on the skin of a susceptible person, and is antitoxinogenic; but it is relatively non-toxic to laboratory animals. It is described both as heat-labile (Stock 1942) and heat-stable (Hottle and Pappenheimer 1941), which suggests either two components, or a substance differing in properties according to the method of purification (Stock and Verney 1952). Culture filtrates of *Str. pyogenes* also contain thermolabile (55° C. for 30 min.) hæmolysins and leucocidins which, like the erythrogenic toxin, are toxic only in large doses. The hæmolysin kills susceptible rabbits within 24-36 hours with an associated hæmoglobinuria and characteristic evidence of intravascular hæmolysis at necropsy (McLeod and McNee 1913, Channon and McLeod 1929).

One implication which has sometimes—though without justification—been attached to the term "exotoxin" is very probably misleading. Toxins of this type have been described in words which suggest that they are of the nature of active secretions of the bacterial cells. There is little definite evidence that this is so. Though some exotoxins, as that of *Cl. welchii*, are produced in high concentration during the phase of active bacterial growth, others, such as diphtheria, tetanus and Shiga toxins, only attain a high concentration in a fluid culture when the phase of active growth has passed, and the majority of the bacteria present are dead or dying. How far autolytic processes are necessary for the liberation of toxin is uncertain. In *Cl. tetani*, large amounts of toxin appear to leave the cells during autolysis (Stone 1954). Morton and Gonzalez (1954) have shown that the toxin of a culture of diphtheria bacilli with

their results indicated a very much higher concentration of toxin in the cells, though the absolute amount of toxin in the fluid was the greater.

Most of the studies on bacterial toxins have been made with crude reagents—filtrates, autolysates or extracts of fluid cultures,—which quite certainly contain many substances other than the toxins. Moreover, it is now clear that a single species of bacterium may elaborate a number of different exotoxins and endotoxins. For instance, filtrates of *Cl. welchii* Type B may contain at least five distinct exotoxins, and two major endotoxins have been isolated from virulent typhoid bacilli.

Constitution of Toxins.—Several exotoxins have been prepared in a purified

LABORATORY METHODS FOR THE STUDY OF BACTERIA¹

The study of bacteria in the laboratory by the various procedures of isolation in pure culture, cultivation, microscopic examination, and characterization by biochemical and immunological methods necessarily begins before detailed consideration of their nature and properties can be completed. The basic laboratory procedures are summarized here in didactic form, and their rationale will become apparent later.

STERILIZATION

The determination of the character and properties of a given species of bacteria is necessarily based on their study in pure culture, *i.e.*, their separation from other microorganisms. Since bacteria and other microorganisms such as fungi are ubiquitous, all material coming in direct contact with the bacteria under study must be subject to preliminary sterilization. The sterilizing agent most commonly used is heat, dry or moist, but occasionally other methods, such as filtration of liquids through bacteria-proof filters, are desirable.

Glassware and Instruments. The usual glassware includes flasks, petri dishes, test tubes and pipettes which must, of course, be scrupulously clean. Flasks and tubes are plugged with nonabsorbent cotton which prevents entry of bacteria after sterilization, and a cotton plug is inserted in the mouth end of the pipettes. Petri dishes and pipettes may be placed in cans with covers or wrapped in paper to maintain sterility. Surgical instruments are wrapped in paper or towels, syringes separated and wrapped in paper, hypodermic needles placed in plugged test tubes, and other equipment similarly prepared.

Sterilization is effected in a hot air oven, electric or gas fired. The material to be sterilized is placed in the oven without crowding and the temperature raised to 170° to 180° C. and maintained for a period of not less than two hours. If appropriate temperature control equipment is not available, a slight browning of the cotton plugs is taken as indicative of sterilization, but this is a doubtful procedure.

In some instances sterilization by heating to dull red in a bunsen flame is exceedingly useful as in the flaming of forceps tips, the platinum or nichrome wire needles and loops used for transferring bacteria, and the lips of test tubes before and after transfer of bacterial culture.

Surgical instruments, hypodermic needles and syringes, and similar equipment may be sterilized with respect to the vegetative forms of bacteria by boiling in water or 1 per cent bicarbonate solution for 3 to 5 minutes, but this does not suffice to destroy the spores of bacteria and fungi.

Culture Media and Other Liquids. Material containing water cannot, of course, be sterilized by dry heat, but moist heat is a more effective sterilizing agent in any case.

of the difficulties in determining the mode of action of bacterial toxins is the separation of the secondary effects due to tissue breakdown from the primary effects of direct intoxication by some constituent of the bacterium. The comparatively rapid action of some exotoxins, especially when administered intravenously, suggests that we are dealing with a primary effect. If, however, the effect is delayed for a few hours, it is impossible on *a priori* grounds to distinguish a latent period due to a slow penetration of toxin into, or a slow accumulation of toxin in, primarily susceptible tissue, from a latent period during which secondary toxins are being elaborated in primarily intoxicated tissues. The difficulty will probably be resolved by precise physiological and biochemical studies on the action of pure preparations of bacterial toxins. It must be realized, however, that the conspicuous effects that have been obtained in short-period observations of animals injected with fairly large doses of toxin may have little direct bearing on the relatively slow process of intoxication that occurs in natural bacterial infections (Wright 1942).

To postulate that toxins interfere with some essential metabolic processes in the animal tissues is merely another way of saying they are toxic. They may, for example, act by poisoning enzymes, rendering substrates insusceptible to enzymes, altering the quantity or quality of the products of certain enzyme processes, or by disrupting structures or surfaces upon which a balanced interrelation of metabolites depends. Peters and Cunningham (1941) could find no change in heart muscle dehydrogenase systems treated with diphtheria toxin. The toxin is reported to affect carbohydrate metabolism in rabbits by over-secretion of adrenaline; and in liver tissue of intoxicated rabbits the storage of glycogen and synthesis of carbohydrate is diminished (see Cross and Holmes 1937).

Another approach to the problem was made by Pappenheimer. Starting from the observation that in artificial culture the concentration of iron for optimal toxin and porphyrin formation is low, and that with more iron the respiratory enzyme cytochrome *b* was formed instead (Pappenheimer and Hendee 1947, 1949), he postulated that the toxin was the protein part of cytochrome *b* and as such might interfere with oxidase systems in mammalian cells that depend on iron enzymes. *In vitro* tests of this hypothesis proved inconclusive. But in the *Cecropia* silk worm, which is susceptible to the toxin in the larval and imago stage, metamorphosis to the pupal stage is accompanied by a 70-fold increase in resistance. Both larva and imago have high succinoxidase activity, which is dependent on cytochrome enzymes, but in the pupa this is apparently confined to the intersegmental muscles; and only these muscles are paralysed in pupae given toxin. Moreover, development of the pupa into imago is inhibited by small doses of toxin (Pappenheimer and Williams 1952). In the silkworm, at least, it appears that diphtheria toxin acts by blocking the synthesis of components of the cytochrome system. Conclusive evidence of a similar action in mammals is still lacking.

The mode of action of *Sh. dysenteriae* toxin is not known; but Penner and Klein (1952) record experiments indicating a direct action on the brain. They crossed the circulation of pairs of dogs, so that the systemic circulation of one supplied the cerebral circulation of the other, and *vice versa*. The absorption of toxin into the systemic circulation of one dog produced no intoxication in that animal; signs of general intoxication, on the other hand, appeared in the other animal, whose brain alone had been exposed to the toxin-containing blood of the first.

In order to explain the extraordinary potency of exotoxins in terms of the small number of molecules required to kill, it is suggested that they may act by preventing enzyme synthesis (see Pappenheimer 1948). One exotoxin, however,—the α -toxin of *Cl. welchii*—is almost certainly enzymic. Filtrates of *Cl. welchii* cultures, for example, will in large doses inhibit the aerobic oxidation of succinate by guinea-pig tissue (Wooldrige and Higginbottom 1938) and have varied actions in the whole animal, ranging from direct action

Intermittent Sterilization. As indicated above, boiling water does not provide a high enough temperature to bring about complete sterilization. Certain kinds of liquid culture media, however, are affected unfavorably by moist heat at higher temperatures, and these may be sterilized by intermittent sterilization. This is based on the assumption that the vegetative cells of microorganisms are destroyed at 100°C in the presence of water, and that the surviving spores will germinate and these vegetative cells will be destroyed in turn by exposure to 100°C . In practice the material to be so sterilized is exposed to free flowing steam in an Arnold sterilizer for 30 minutes, removed and incubated until the next day and the steaming repeated, and a third incubation and steaming is included as a safety factor so to speak. The method is not too successful in that spores not infrequently show a delayed germination, and spores of obligate anaerobic bacteria may not germinate. Sterilization by the intermittent method is, however, seldom required.

Autoclave Sterilization. The most efficient method of sterilization is that effected by steam at temperatures above 100°C . which may be produced when the steam is under pressure. The devices employed in the use of steam under pressure include the common pressure cooker, autoclaves of various designs and dressing sterilizers, which are autoclaves

too tightly in the autoclave. The temperature is raised to 120°C . and maintained for a period of time depending on the material to be sterilized. As short a time as 15 minutes

Sterilization by Filtration. It is often desirable to sterilize solutions such as bacterial cultures in liquid media, solutions of substances relatively unstable to heat such as certain sugars and the like, without subjecting them to heat at sterilizing temperatures. These may be sterilized by filtration through filters of such fine porosity that bacteria are held back. The method of sterilization by filtration is particularly useful in obtaining soluble bacterial products such as toxins.

Several kinds of bacteria proof filters are available. The Berkefeld filters are made of

is made in graded porosities, L1, L2, L3, etc., and of these the L3 is roughly equivalent to the Berkefeld N. The Seitz or Seitz-Werke filter is made of metal and the filtering element is an asbestos pad which is the equivalent of the Berkefeld N filter. Sintered glass filters are available in a variety of porosities which in the pyrex filters are designated C (coarse), M (medium), F (fine) and UF (ultrafine). The UF filter is bacteria proof while the others are not.

Filtration is accomplished by a pressure differential which may be obtained by positive pressure on the liquid to be filtered, or negative pressure on the filtrate, and usually a vacuum filter is used. The vacuum filter is used

PREPARATION OF CULTURE MEDIA

The nutritive requirements of bacteria vary greatly, some will grow readily in the so-called synthetic media containing inorganic salts, including an am-

It is possible that adrenal stimulation and damage may play some part in the production of these effects (see Evans and Zeckwer 1927, Olitzki, Avinery and Koch 1942). In *Sh. dysenteriae* Olitzki and his colleagues (Olitzki, Leibowitz and Berman 1937, Olitzki and Avinery 1937) distinguish a toxic fraction and separate fractions producing leucopænia, glycaemia and hypothermia respectively. It is not certain how far their fractions were made up of complete endotoxin, and of breakdown products therefrom.

Other Active Constituents or Products of Bacteria.

We have already noted certain specific or non-specific biological characteristics of toxins; and many others, like the hæmolysins and leucocidins of *Staph. aureus*, could be instanced. In this section we are concerned with bacterial products that are not particularly toxic in themselves, but act upon animal tissues and fluids, or upon substrates known to occur in animal tissues, in such a way as to suggest that they may influence the course of a bacterial infection. As with the toxins, the proof of their auxiliary powers in infection depends ultimately on demonstrating their activity in the natural infection, either directly, or indirectly, by the protective action of antibodies and other specific inhibitors of the substance in question. In this group of bacterial substances particularly, it is to be emphasized that the mere association of a biochemical property and pathogenicity, either in a bacterial species or in the more virulent strains of a pathogenic species, is not by itself evidence that the property is concerned in infection by the species. As we have seen in the chapter on variation, latent activities of bacteria can be made manifest by the provision of suitable substrates, and though a virulent bacterium may exhibit an activity because a suitable substrate is present in the tissues of its host, it does not necessarily follow that the virulence depends on the capacity to attack the particular substrate; the capacity may in this respect be purely accidental.

Coagulase.—Among the staphylococci, the more pathogenic *Staph. aureus* produces a coagulase that accelerates the clotting of human and rabbit plasma (Much 1908, Gross 1931a, b, Gengou 1933, Chapman *et al.* 1934). Since the formation of fibrin clots is a striking feature of *Staph. aureus* lesions in man and animals, it is natural to relate the two features. Nevertheless, Menkin and Walston (1935) could find no evidence that staphylocoagulase was concerned in the production of the staphylococcal lesions.

Susceptibility of plasma to coagulase varies with the species (see also Gerheim and Ferguson 1949). Guinea-pig plasma, for example, is usually not affected. Smith and Hale (1944) found that susceptibility depended on an activator in plasma; and that activator from susceptible human or rabbit sera made guinea-pig plasma susceptible. Although its identity is not fully established, the activator resembles prothrombin in many respects (see Tager and Hales 1948a, Duthie and Lorenz 1952).

The auxiliary rôle of coagulase is based on the association of coagulase α -toxin production in clinically infecting strains of *Staph. aureus*, (see Schwabacher *et al.* 1945); the association of species insusceptibility to staphylococcal infection, as in fowls, rats and mice, with insusceptibility to coagulase (Hale and Smith 1945); the fact that coagulase-production confers resistance to phagocytosis on strains of *Staph. aureus* tested in the presence of a coagulable plasma (Hale and Smith 1945); and the increase in virulence of coagulase-producing *Staph. aureus* for mice, when injected with a coagulable plasma from another species (Smith *et al.* 1947). Coagulase is antigenic (Tager and Hales 1948b), and antibody to it occurs in the serum of patients with chronic staphylococcal infections (Duthie and Lorenz 1952). Finally, according to Duthie (personal communication), there are two coagulase factors, one bound to the cell, the other free. The bound factor is liberated on autolysis of the cell, acts directly on a susceptible fibrinogen, and is neutralized by

monium salt, together with a simple organic compound such as glucose or asparagin as a source of carbon and energy. In general, bacteriological media have been developed on a trial and error basis about a basic nutrient medium containing peptone and the water-soluble material, largely extractives, of muscle tissue. The source of the latter may be commercial preparations of meat extract but a somewhat better medium is obtained if these substances are extracted from fresh meat, beef or veal for the most part.

The basal nutrient solution may be modified in a variety of ways. Thus, it may be solidified by the addition of gelatin or agar, or enriched with serum, ascitic fluid, defibrinated blood and the like to support the growth of the more fastidious bacteria. Various sugars may be added, together with an acid-base indicator, for the determination of the fermentative properties of bacteria; nitrate or tryptophane may be added to test for ability to reduce nitrate to nitrite, form indol from tryptophane, etc. Or culture media may be prepared which are differential and accentuate physiological differences, and specific inhibitory agents may be added to give selective media which allow the growth of some kinds of bacteria and suppress that of others. These various characteristics may be combined, of course, as in the case of an enriched medium which is both selective and inhibitory. In consequence, there is a great variety of bacteriological culture media, but they are in large part interrelated modifications of a basal nutrient solution. The constituents and method of preparation of a few of the commonly used culture media may be reviewed here.

Basal Media

Meat Extract Broth and Agar. The medium usually has the following composition:

meat extract	3 gm.
peptone	5 gm.
(NaCl	5 gm.)
agar	15 gm.)
distilled water	1000 ml.

It is necessary to include the sodium chloride if the medium is to be used for serological work or enriched with blood, and the agar is omitted if a liquid medium is desired. The ingredients are dissolved in water. Agar has the property of not dissolving or of its gels the temperature heat it in the y acid and the

reaction is adjusted to pH 7 by the addition of alkali.

Meat Infusion Broth and Agar. The infusion media differ from the extract media in that the extractives are obtained by infusion of fresh meat, but otherwise the composition is the same, viz.

extract of 400 to 600 gm. fresh lean beef or veal	
peptone	5 gm.
(NaCl	5 gm.)
agar	15 gm.)
distilled water to	1000 ml

The meat is ground in a meat chopper, suspended in 1 liter of distilled water, and infused overnight in the refrigerator. The following morning the fat is skimmed off with absorbent cotton and the infusion squeezed through muslin and made up to 1000 ml. The other ingredients are added, the reaction adjusted to pH 7, the solution heated to 100° C. for about 20 minutes, regardless of whether agar is used, filtered through coarse paper and made up to 1000 ml. The heating is for the purpose of coagulating the tissue proteins dissolved during infusion so that they can be removed by filtration.

been found in members of the *Bacterium*, *Pasteurella*, *Rickettsia*, *Bacillus*, *Brucella*, *Vibrio*, *Haemophilus* or *Neisseria* groups (see also Ungar and Bacharach 1942).

The identity of certain mammalian and bacterial diffusing factors with the enzyme hyaluronidase was first suggested by Chain and Duthie (1940). The parallelism is not complete (Hobby *et al.* 1941, Meyer *et al.* 1941), but it is clear that most bacterial spreading factors owe their activity to one or more enzymes catalysing different stages in the breakdown of polymers of hyaluronic acid (McClellan 1941, McClellan and Hale 1941, Rogers 1945, 1946, 1948, Humphrey 1946). The intercellular ground substance of mesodermal tissue contains hyaluronic acid in a viscous polymerized form, and it appears that the enzyme hydrolyses the viscous polymer and thus permits the ready diffusion of fluid through the intercellular spaces.

It is evident that the addition of hyaluronidase to the inoculum enhances the virulence of the contained organisms in certain experimental infections. In others, it has no effect, or decreases their virulence. As we have already noted, a decrease in virulence is explicable on the grounds that the inoculum is dispersed. Its efficacy also depends on the pressure engendered at the site of inoculation when the injection is made (Hechter 1947). Given, for example, in the allantoic sac of the chick embryo, hyaluronidase does not affect influenza viral infections (see Fastier 1951, Henneberg and Ortmann 1951). In natural infections, therefore, we might expect a bacterial hyaluronidase to promote the spread of infection in oedematous lesions where the exudate is under increased pressure, provided that the spread of the invader took place into susceptible and not into resistant tissue. McClellan and his colleagues (1943) detected large amounts of hyaluronidase in well-established local lesions by *Cl. welchii*, *Cl. septicum* and certain strains of *Cl. oedematiens*, and it is reasonable to postulate that in such circumstances the spread of the hyaluronidase-producing organisms would be facilitated by the enzyme. But it may be argued that, in the very early stages of a natural infection when the growing bacterium is present in small numbers, hyaluronidase, by promoting a rapid dispersal of the bacteria through healthy tissues, would expose invading organisms more thoroughly to the lethal action of the tissues (see, *e.g.*, Dalgaard-Mikkelsen *et al.* 1950).

The production of hyaluronidase, like that of coagulase and fibrinolysin, is associated with virulent species of bacteria, and to some extent with the virulent strains within a species. The association between invasiveness and hyaluronidase production is by no means complete. Thus some highly invasive strains of *Cl. welchii* produce none (Evans 1943b); and the same is true of certain types of *Str. pyogenes* obtained from severe human infections (Crowley 1944) and of streptococci that prove pathogenic for chick embryos and laboratory animals (Russell and Sherwood 1949, Sherwood *et al.* 1952). Some Group A streptococci, which depend in part upon the presence of a mucoid capsule containing hyaluronic acid, also secrete hyaluronidase (Pike 1948). That they may thereby decrease their own virulence is evident from the protective and curative effect of hyaluronidase in experimental infection by streptococci possessing hyaluronate capsules (Kass and Seastone 1944, Rothbard 1948). Bacteria like *Brucella melitensis* and *tularensis* and *Pasteurella pestis* which are highly invasive in the accepted sense of the word, produce no hyaluronidase.

and the tubercle bacillus (Bergqvist and Packalen 1948)—an effect attributed to hyaluronidase. Humphrey (1944) found no relation between *in vitro* hyaluronidase production by pneumococci and the severity of the human infection they caused, though in 20 strains Grumbach and Kradholfer (1945) related high productivity with rate of spread of the lung lesions, as measured by X-rays. Thompson (1948) found the anti-hyaluronidase response in pneumonia to be directly related to the severity of the disease. This suggests considerable *in vivo* production; but the high productivity may have been merely the result, and not the cause, of extensive bacterial proliferation.

From the foregoing evidence we see that the auxiliary pathogenic rôle of hyaluronidase in natural infection is largely conjectural. A more convincing proof might be obtained

Media for Biochemical Tests

Sugar Broths. The sugar broths are meat extract or meat infusion broth to which the required carbohydrate has been added to a concentration of 1 per cent. Unless the carbohydrate is stable to autoclave sterilization, it must be sterilized separately by filtration in concentrated solution and added aseptically to the sterile broth. It is customary to add an acid base indicator dye, bromthymol blue or bromcresol purple, and the medium may be dispensed in culture tubes containing an inverted vial that fills during sterilization and serves to collect gas evolved in the fermentation of the sugar.

Nitrate Broth. To test for ability to reduce nitrate, bacteria are cultured in nitrate broth, infusion or extract broth containing 0.1 per cent KNO_3 . The culture is tested for the presence of nitrite with sulfanilic acid and α -naphthylamine reagents.

ml
par
by filtration through absorbent cotton.

To test for nitrite add 2 ml. of each of the two reagents to 3 to 5 ml. of culture, the development of a rose color, the nitroso reaction, indicates the presence of nitrite.

Tryptophane Broth. The test for the production of indol from tryptophane can often be
tryptone contains adequate amounts of the amino acid

The presence of indol is tested for after 2 to 4 days' incubation of the culture by layering a small amount of Ehrlich's reagent on the surface of the culture, the development of a red color at the interface indicates the presence of indol. If a color does not appear within 1 minute, add a small amount, equal to that of the Ehrlich reagent, of saturated aqueous solution of potassium persulfate.

Ehrlich's reagent consists of 4 gm. of *p*-dimethylaminobenzaldehyde dissolved in a mixture of 380 ml. of ethyl alcohol and 80 ml. of concentrated hydrochloric acid.

Lead Acetate Agar. This medium is meat extract or meat infusion agar containing 0.05 per cent basic lead acetate and dispensed in culture tubes without slanting. It is usually prepared by mixing equal amounts of sterile double strength basal medium, i.e., containing double amounts of the ingredients, and sterile 0.1 per cent aqueous solution of basic lead acetate.

The medium is inoculated by stab and the production of hydrogen sulfide from the sulfur-containing amino acids of the peptone is indicated as a blackening or browning of the medium.

Meat Extract and Meat Infusion Gelatin. These media are the basal media solidified

by chilling. Gelatin liquefaction sometimes occurs slowly and cultures should be retained for not less than two weeks unless positive earlier.

Milk. Milk is an adequate culture medium for many bacteria without modification other than the addition of an indicator, bromcresol purple or litmus in an amount of 5 ml. of a 0.25 per cent alcoholic solution per liter. Fresh skim milk may be used, but skim milk powder is usually more convenient. One liter of medium is made from 150 gm. of milk powder, first rubbed up in a mortar and then diluted to volume. Medium prepared from powdered milk may be autoclaved, but that from fresh milk is best sterilized by the intermittent method.

Because of its protein and carbohydrate content milk cultures undergo a variety of biochemical changes, viz.:

(1) The development of an alkaline reaction, usually after 3 to 4 days' incubation.

act as a powerful stimulus. In some cases the vigorous response evoked is injurious to the host, so that a bacterial product which possesses little or no toxicity for a normal animal may be highly toxic for an animal which has become sensitized by infection. The well-known tuberculin reaction in the guinea-pig is a case in point.

Some Illustrative Examples.

We have described above the various bacterial factors which, so far as we know at the moment, are certainly, probably, or possibly concerned in the pathogenesis of infective diseases.

A few illustrative examples may serve to emphasize the more important points.

Botulism may be placed at one end of the scale, as an example of a pure toxæmia. It is not a true infection, but is closely analogous to the effects that may follow the consumption of such vegetable poisons as those produced by certain fungi or plants. There is no certain evidence that *Cl. botulinum* ever invades the tissues, or multiplies in them. Man is affected by eating food in which the organism has grown and produced its toxin.

Tetanus is one step removed, in that the bacillus must gain access to the tissues and multiply in them in order to produce its toxic effects; but its invasive powers are very slight. It usually gains access to the body as the result of some mechanical injury, and the local multiplication of the organism is often assisted by gross injury to the tissues, by the injurious effects of some associated infection, or by the presence of some chemical agent which damages the local defensive tissues.

Diphtheria takes us a little further along the scale. The bacillus is capable of implanting itself in the faucial region, and of spreading from host to host, by ordinary droplet infection or in similar ways. It has relatively slight but quite definite invasive powers. It causes a characteristic local lesion, and may occasionally invade other tissues and organs. Its lethal effect, however, depends almost entirely on its activity as a toxin producer.

The hæmolytic streptococcus combines the activities of a typically toxigenic organism with those of the highly invasive group of bacteria. It produces a relatively heat-stable toxin, with a characteristic action on the skin, which is the cause of the typical toxic rash in scarlet fever; but it also invades the tissues freely. Indeed, highly virulent strains of the organism are among the most rapid and vigorous tissue-invaders with which we are acquainted in human pathology. In relation to this tissue invasion it is probable that the leucocidin, and perhaps the hæmolysin, fibrinolysin and hyaluronidase produced by pathogenic streptococci play a significant part.

As we move along the scale we meet a variety of organisms whose pathogenicity seems to depend entirely on their power of spread and multiplication in the tissues, and whose more important effects seem to be localized to the areas in which such multiplication occurs. These differ among themselves in selective localization, and in the tissue reactions to which they give rise. Thus, staphylococci most commonly cause localized suppurative lesions, with a varying degree of lymphatic spread, which may sometimes be rapid and extensive. More rarely they give rise to a generalized infection of the pyæmic type, in which the cocci are carried from the original focus of infection in detached fragments of an infected thrombus. Here again, leucocidin, coagulase, and perhaps hæmolysin, fibrinolysin and hyaluronidase, may play a part in determining the type of lesion in any particular case.

Finally, there are organisms, such as the tubercle bacillus, the leprosy bacillus

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- (2) The development of an alkaline reaction with precipitation of the casein as a rennet curd, with or without reduction of the indicator.
- (3) The development of alkalinity and rennet curd precipitation, followed by peptonization or digestion of the curd, resulting in a clearing and brownish discoloration; the indicator is usually reduced by the time digestion is apparent.
- (4) Acid formation, usually in 24 hours, with or without reduction of the indicator.
- (5) Acid formation and precipitation of the casein, usually with reduction of the indicator.

Enriched Media. Basal infusion media may be made richer by the use of special peptones, such as neopeptone and proteose peptone, and the addition of small amounts of glucose and phosphate buffer. In addition, fluids such as serum, ascitic fluid or defibrinated blood may be added.

Blood Agar The most common and most useful enriched medium is blood agar—veal or beef infusion base to which defibrinated whole blood is added. The blood is usually taken from the rabbit, sheep or horse under sterile conditions and defibrinated as it is drawn by shaking with glass beads. The sterile infusion agar base is liquefied by heating, cooled to about -45°C , the sterile blood added to 10 per cent concentration, and the medium dispensed before it solidifies, usually in petri dishes or as slants in culture tubes. The entire operation is carried out under sterile conditions and the medium incubated for 24 hours to detect contamination, and then stored in the refrigerator. This medium is rich enough to support the growth of almost all the fastidious pathogenic bacteria and has the advantage that hemolysis is shown directly in the cultures.

Cystine Blood Agar This is simply blood agar medium further enriched by the addition of 0.01 per cent cystine and 1 per cent glucose to the basal medium prior to the addition of blood as above. It is useful primarily in the cultivation of *Pasteurella tularensis*.

Chocolate Agar. This medium is heated blood agar and is particularly useful for the cultivation of the gonococcus and meningococcus. Formulae vary somewhat, some workers preferring a beef heart infusion (see below) instead of muscle tissue infusion and proteose peptone instead of peptone. The basal infusion medium is enriched by the addition of defibrinated blood as in the preparation of blood agar, but after the addition of the blood, it is heated slowly in a water bath until the medium has a chocolate brown color, if the heating is excessive the blood coagulates and the finished medium will contain clumps of cooked blood, so that the temperature should not rise above 75°C . at the most.

Other Infusions. The use of infusions of tissues other than beef or veal muscle is sometimes desirable for the cultivation of certain bacteria. Beef liver infusion is prepared in the same manner as that described for the other infusions and, for culture of Brucella, for which liver infusion medium is particularly useful, the basal medium is enriched by the addition of 1 per cent egg albumin. Beef heart infusion is also prepared in the same manner as beef muscle infusion and the basal medium completed by the addition of a peptone, sodium chloride, etc.

Löffler's Medium. This medium is used primarily for culture of the diphtheria bacillus and consists of 3 parts of sterile beef serum and 1 part of meat infusion basal medium containing 1 per cent glucose. The serum is added to the sterile base, mixed, dispensed in culture tubes, slanted and sterilized by the intermittent method. Sterilization may be carried out on three successive days in the autoclave at 15 pounds steam pressure without letting the air out, or it may be held at 15 pounds for 15 minutes and the air allowed to escape slowly while maintaining pressure and, when all the air has escaped, holding for an additional 15 minutes, and after sterilization allowing the pressure to fall very slowly. The medium is solid because of coagulation of the serum and sterilization must be carried out carefully to avoid the formation of bubbles.

Blood Culture Medium (Kracker). This is a highly enriched medium containing beef heart infusion and brain suspension and is used primarily for blood culture of bacteria in infections accompanied by bacteremia. Its composition is:

been most extensively studied in Gram-negative bacteria, where they appear to confer on the cell the main serological properties of the virulent S forms.

(6) There are other substances in bacterial cells which are not in themselves toxic, but whose nature—the capacity to induce or inhibit the coagulation of plasma or lymph, to dissolve a fibrin clot, or to increase the local intercellular permeability of tissues—suggests that they may influence the course of the infective process; and it is possible that other enzymes concerned in bacterial metabolism may have a like influence in infection by alteration of the metabolism of the tissues.

(7) The pathogenic rôle of these auxiliary factors, and even of endotoxins that have no specific effect on the host's tissues, is often difficult to establish. In doing so, it is necessary in the first place to define the stage and circumstances of the natural infection in which the presumed auxiliary factor is effective and, if possible, to demonstrate the effect in these circumstances. The association of the factor with virulent strains of an organism, and the antibody response to the factor in the infected host, are of limited value as evidence. When the factor is antigenic, the most convincing evidence of its participation is a curative or protective effect of antibody specific for the factor.

(8) As the result of infection the tissues of the host may become hypersensitive to the infecting bacteria or to their products. Hypersensitivity reactions may be responsible for some of the characteristic phenomena of infective disease.

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heart infusion	750 ml
brain suspension	250 ml
sodium citrate	1 gm
dextrose	10 gm.
peptone	10 gm.
dibasic sodium phosphate	2 gm
sodium chloride	4 gm

The heart infusion is prepared in the same manner as muscle tissue infusion described earlier. The brain suspension is prepared by maceration of 500 gm. of fresh brain in water, straining through a metal strainer and heating slowly to boiling with constant stirring. The heating coagulates the brain tissue and leaves it in a state of fine suspension, it must not be filtered. The other ingredients are added to the mixture of heart infusion and brain suspension, the pH is adjusted to 7.4, the mixture is dispensed in 50 ml. amounts in 100 ml. flasks (to leave room for an inoculum of 10 to 20 ml. of patient's blood) and sterilized by autoclaving. Cultures in this medium are essentially enrichment cultures and should be subcultured on appropriate agar media such as blood agar for streptococci and staphylococci, liver infusion agar for *Brucella*, etc.

Dorset's Egg Medium. This medium is used for the cultivation of the tubercle bacillus and consists of 4 fresh eggs and 25 ml. of 0.85 per cent sodium chloride solution. The eggs are scrubbed with a brush in soap and water, rinsed, dried, put into a wire basket and dipped into 95 per cent alcohol, drained and the remaining alcohol ignited. They are then broken using aseptic technique and the whites and yolks placed in a sterile container. Twenty five ml. of sterile salt solution is added, the whole mixed well with a sterile egg beater, dispensed in culture tubes and slanted, and sterilized by the intermittent method described for Löffler's medium.

Differential and Selective Media. The various differential and selective media have been designed to facilitate the isolation of one kind of bacteria in the presence of many other kinds, by accentuating physiological differences and/or inhibiting the growth of the

fermenting bacteria are yellow while those of the non fermenters are blue. Of the selective agents bacteriostatic dyes are often used, and others include bile, tellurite, etc.

Endo's Medium. This medium consists of meat extract agar to which has been added lactose and Schiff's reagent, basic fuchsin decolorized with sulfite. When the lactose is fermented aldehyde intermediates restore the color to the fuchsin and colonies of lactose fermenting bacteria are red while those of the non lactose fermenters are white. The composition of the medium is

hot melted extract agar	1000 ml.
sodium carbonate, 10% aqueous	10 ml
lactose	10 gm
sodium bisulfite, 10% aqueous	10 ml.
basic fuchsin, 3% alcoholic	10 ml.

The pH of the medium is raised by the carbonate and should be 7.6 to 8.0.

Eosin-Methylene Blue Agar. This medium is sterile meat extract agar to which is added 5 ml. of a 10 per cent solution of lactose, 2 ml. of 2 per cent aqueous eosin and 2 ml. of 0.5 per cent aqueous methylene blue. These solutions are added aseptically to the melted and cooled medium and the mixture is dispensed in sterile petri dishes.

Desoxycholate Citrate Medium. This medium is differential in that it contains lactose and neutral red as an indicator, and selective in that it contains bile salt, and is one of the

been most extensively studied in Gram-negative bacteria, where they appear to confer on the cell the main serological properties of the virulent S forms.

(6) There are other substances in bacterial cells which are not in themselves toxic, but whose nature—the capacity to induce or inhibit the coagulation of plasma or lymph, to dissolve a fibrin clot, or to increase the local intercellular permeability of tissues—suggests that they may influence the course of the infective process; and it is possible that other enzymes concerned in bacterial metabolism may have a like influence in infection by alteration of the metabolism of the tissues.

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most satisfactory and widely used media for the isolation of *Salmonella* and dysentery bacilli. It contains

meat infusion	1000 ml.
peptone	10 gm.
agar	20 gm.
lactose	10 gm.
sodium citrate	20.6 gm.
sodium desoxycholate	5 gm.
lead chloride, 0.35% aqueous	1 ml.
ferric ammonium citrate	2 gm.
neutral red, 1% aqueous	2 ml.

The complete medium is not stable on storage. The peptone and meat infusion are mixed, the pH adjusted to 7.5. The solution is boiled for 3 minutes, lost water added, and filtered through paper. The agar is added to the hot solution together with 5 ml. of N NaOH and it is allowed to stand for 15 minutes, then steamed at 100° C. for 20 minutes. Then the lactose, citrate, desoxycholate and lead chloride are added. This may be stored and remixed for use. Just before use, the agar is heated to 100° C., the ferric ammonium citrate added and the pH adjusted to 7.4 using phenol red as an indicator, and finally the neutral red is added and the medium dispensed in plates. Note that it is not autoclaved.

Bismuth Sulfite Medium (Hajna). This medium is used primarily for the isolation of the typhoid bacillus from fecal specimens and is highly specific for that organism. As originally developed it was difficult to prepare consistently but Hajna's modification is highly satisfactory. It consists of a meat extract agar base containing 0.5 per cent glucose, the bismuth sulfite mixture and brilliant green. The bismuth sulfite mixture is prepared as follows

- (1) Dissolve 80 gm. of anhydrous bismuth sulfite in 400 ml. of hot water with stirring.
- (2) Make a paste of 24 gm. of bismuth citrate in 40 ml. of water, add 12 ml. of concentrated ammonia and stir until a sol is formed. Dilute to 200 ml. with distilled water and mix to give a solution.
- (3) Mix (1) and (2) and add 42 gm. of anhydrous diarsenic sodium phosphate and mix until dissolved.
- (4) Dissolve 4 gm. of ferrous sulfate in 50 ml. of distilled water containing 2 drops of concentrated hydrochloric acid, and add 40 ml. of this solution to mixture (3). Mix and boil gently for about 2 minutes until slate gray in color. This is the bismuth sulfite mixture and it is stable for about 2 months.

For use add 70 ml. of the bismuth sulfite mixture and 4 ml. of a 1 per cent aqueous solution of brilliant green to each 1000 ml. of the hot melted agar base and autoclave for not more than 10 minutes, and dispense in petri dishes. The complete medium may be kept for perhaps 2 weeks in the refrigerator without autoclaving, and should be autoclaved just prior to dispensing.

Tetrathionate Enrichment Broth. Nutritive enrichment broths are used for preliminary culture of specimens followed by subculture on selective differential media. Tetrathionate broth and selenite F broth (see below) are very useful in the isolation of *Salmonella* from fecal specimens. The active agents in tetrathionate broth are bile salts, brilliant green and tetrathionate, the last formed by oxidation of thiosulfate with iodine just prior to inoculation. Its composition is as follows:

proteose peptone	5 gm.
bile salts (Bacto)	1 gm.
distilled water	1000 ml.
calcium carbonate	10 gm.
sodium thiosulfate	30 gm.
brilliant green, 1% aqueous	11 ml.
iodine, 25% aqueous	25 ml.
potassium iodide	per 100 ml.
water to	25 gm.
	20 gm.
	100 ml.

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The peptone and bile salts are dissolved in water and calcium carbonate added, and autoclaved. The sodium thiosulfate and brilliant green are added to the sterilized medium and it is dispensed in tubes or small flasks. Just prior to inoculation add the iodine solution in the proportion of 2.5 ml per 100 ml.

Selenite F Broth. This medium contains selenite as an inhibitory agent and its composition is:

sodium hydrogen selenite	4 gm.
peptone	5 gm.
lactose	4 gm.
sodium phosphates (anhydrous)	10 gm.
distilled water	1000 ml.

It is necessary to determine the proportions of acid and basic phosphates which will give a final reaction of pH 7.0 to 7.1 with the peptone and the lot or brand of selenite used. The ingredients are dissolved in warm water, and then brought to a boil and dispensed. The medium is not autoclaved.

ml. of infusion base. It is used for culture of diphtheria bacilli.

Petragnani's Medium This is a medium used for culture of tubercle bacilli and contains malachite green to inhibit the growth of gram positive bacteria and molds. Its composition is:

milk	900 ml.
potato flour	36 gm.
peptone	6 gm.
pieces of potato, size of an egg	6
eggs	24
egg yolks	6
glycerin	72 ml.
malachite green, 2% aqueous	60 ml

The potatoes are cut into thin slices, the milk, potato flour and peptone added, and the whole cooked in a double boiler for 2 hours with stirring. The eggs and egg yolks are broken together, the glycerin and malachite green added, and the mixture shaken well. The milk potato mixture is cooled to 50° C, the egg glycerin-dye mixture added and mixed well, and the whole is filtered through gauze, dispensed into tubes, slanted and sterilized by the intermittent method described for Löffler's medium.

MICROSCOPIC EXAMINATION OF BACTERIA

The direct observation of bacteria is an essential part of their study. Characteristics such as the shape and grouping of the cells, the presence or absence of structures such as capsules, flagella and spores, the reaction to differential stains, and the like are of considerable differential significance.

Motility. Some bacteria are motile by virtue of organs of locomotion, flagella, to the cell. Motility can be observed if the living bacteria are mounted in a drop of liquid on a coverslip inverted over a hollow ground or depression slide. Motility is to be differentiated from brownian movement, the latter is a dancing irregular movement, while motile bacteria move across and in and out of the microscopic field, and the movement may be surprisingly rapid.

were dead within 30 minutes. On the palmar surface of the clean hand *Bact. coli*, *Salm. typhi* and *Salm. enteritidis* could not be detected after 10 minutes, though in certain regions, under the nail-tip and along the lateral margins of the finger nails, the bacteria persisted longer. There was a rapid initial reduction of *Staph. aureus* on the palmar surface of the hand, but small numbers persisted for long periods. The skin of dirty hands had less self-sterilizing power, the reduction of *Salm. enteritidis* being 5 per cent. in 20 minutes; after washing the reduction was 100 per cent. in 20 minutes. Fifteen minutes after the death of the skin, most of the bactericidal action for *Bact. coli* had disappeared.

It should be noted that not only may bacteria be killed, but viruses may be inactivated on skin. Influenza virus, for example, in one series of experiments, could not be detected 10 minutes after application to the human skin (U.S. Navy Medical Bulletin 1943); though in another series (Parker and MacNeal 1944) suspensions of virus in the allantoic fluid of the chick survived 45 to 50 minutes on the skin of the hand.

Arnold and his colleagues attach considerable importance to the effect on bacteria of the pH of the skin, which is usually acid (5.8-5.2, see Schade and Marchionini 1928). Marchionini and others (1938), using a skin electrode, found the greater part of the skin surface was acid, the pH in some spots being as low as 5 or even 3. There were alkaline areas in the axillæ and groins, between the toes and on parts of the foot, which they regarded as gaps in the "acid coat" that constitutes a defence against infection of the skin. The flora of skin was scantier in the acid areas and, in these, applied *Chromobacterium prodigiosum* disappeared more rapidly than in the alkaline areas. After induced sweating the skin increased in acidity, and was more bactericidal. Bergeim and Cornbleet (1943) attribute the acidity to the lactic acid excreted in sweat, and to the volatile acids formed from it by bacterial action. The former is active in regions where the pH of the skin is 5.3 or less; at a higher pH, it dissociates and loses much of its bactericidal power. The latter are active at a pH of 5.3 to 6.2, and will consequently be effective in the more alkaline areas of the skin.

The readiness with which *Str. pyogenes* is killed on the skin has been partly elucidated by Burtenshaw (1938, 1942) (see also Williams *et al.* 1943) who extracted, from normal living or dead skin, substances resembling long-chain fatty acids and soaps that were markedly bactericidal for *Str. pyogenes*, but not for *Staph. aureus*.

Hellat (1948) largely discounts Arnold's hypotheses. He found rapid death on the skin of *Pr. vulgaris* and *Bact. coli*; death within a day of *Ps. pyocyanea* and *Chr. prodigiosum* and within a few days of hemolytic streptococci; and prolonged survival of *Staph. aureus* and enterococci. For most of his bacteria, desiccation was a sufficient explanation. The survival of *Staph. aureus*, and its habitual presence on normal skins (see Chapter 67), he suggests are due to its capacity for multiplying in water-poor substrates (see also Rebell *et al.* 1950). Ricketts and his colleagues (1951) confirmed the rôle of desiccation in the death of *Ps. pyocyanea* and *Bact. coli*, which survived for long periods on moist skin. They identified oleic acid as a major constituent of streptococcicidal fatty acid in the skin. Together with desiccation, this acid likewise appeared to play a large part in killing *Staph. aureus*.

Fleming (1922, 1929, 1932) recorded the presence in tears, in nasal secretion, and in many tissues including the skin, of a relatively thermolabile substance which, in high dilution, causes the lysis of certain non-pathogenic bacteria, and in particular of an organism to which he gave the name of *Micrococcus lysodeikticus*. This substance, lysozyme, is an enzyme (Meyer *et al.* 1936, Abraham 1939) which appears to initiate lysis by splitting certain polysaccharide components of susceptible bacteria (Epstein and Chain 1940). It is active against some strains of staphylococci, and of intestinal streptococci of human origin. In high concentration it acts, though less energetically, on other pathogenic bacteria. It is natural to inquire whether it plays any part in the self-disinfecting action of the human skin. The evidence is conflicting. According to Colebrook (1930) many of the bacteria that are rapidly removed are not susceptible to its action. *Micrococcus lysodeikticus* itself disappears less rapidly from the skin than such species as *Str. hemolyticus*,

For demonstration of motility the culture should be young, not more than 18 hours old. Bacteria from cultures on agar media must be suspended in saline, while broth cultures may be used directly. A drop of suspension or culture is placed in the center of a cover slip. The depression in the hollow ground slide is lined with petrolatum on the surface around the depression, and inverted over the cover slip. The cover slip sticks to the slide and evaporation is prevented by the seal. The preparation is turned over and examined under the oil immersion lens. Unstained bacteria are difficult to see and the diaphragm should be closed to a small aperture, it is best to focus first on the edge of the drop.

Staining. Bacteria may be stained or dyed with aniline dyes, more readily with the basic dyes.² Staining may be with a single dye, or simple stain, most commonly crystal violet, methylene blue or basic fuchsin, with mixed dyes or polychrome stains, or by differential methods based on the relative affinity of different bacteria or different structures of the bacterial cell for the stains used. The nature of the staining process has been a matter of considerable controversy, in large part as to whether it is fundamentally a physical or chemical process. It is now generally agreed that it is chemical in nature and the mechanism an ionic interchange between the basic dye and the acidic portions of the protoplasm—nucleic acids and their compounds—with the formation of insoluble dye-nucleotide compounds that do not diffuse out of the cell.

Stain Solutions. The composition of stain solutions varies considerably in the literature. The several, frequently somewhat indefinite, formulae have been interpreted by the Committee on Biological Stains and are given here in the emended form recommended by that Committee. In older formulae the dye content is often given as ml. of a saturated alcoholic solution, and the accompanying table gives the solubilities of those most commonly used, in water and in 95 per cent alcohol.

SOLUBILITIES OF COMMON BACTERIOLOGICAL STAINS²

Color Index Number	Name	Per Cent Soluble in	
		Water	95% Alcohol
655	Auramine O	0.74	4.49
681	Crystal violet (chloride)	1.68	13.87
676	Fuchsin, basic (pararosanilin hydrochloride)	0.26	5.93
657	Malachite green (oxalate)	7.60	7.52
922	Methylene blue (hydrochloride)	3.55	1.48
841	Safranin	5.45	3.41
925	Toluidine blue O	3.82	0.57

Löffler's Alkaline Methylene Blue

Solution A.

methylene blue (90% dye content) 0.3 gm.
ethyl alcohol (95%) 30 ml.

Solution B.

ddute KOH (0.01% by weight) 100 ml.

The two solutions are mixed in the above quantities.

Ziehl's Carbol-Fuchsin

Solution A

basic fuchsin (90% dye content) 0.3 gm.
ethyl alcohol (95%) 10 ml.

² See *Biological Stains*. Prepared by the Commission on the Standardization of Biological Stains, 11. J. Conn, chairman. 3rd ed. Geneva, N. Y. 1940.

upper part of the duodenum to the action of the gastric juice, with its high hydrogen-ion concentration. It has long been recognized that the gastric secretion imposes a barrier to the passage of bacteria from the mouth to the intestine, but the careful studies of Arnold and his colleagues (Arnold 1926, 1927, 1928, 1929, Arnold and Brody 1926a, b) have placed our knowledge on a more detailed basis. By fixing various segments of the gastro-intestinal tract of the dog to the skin of the abdomen they were able to withdraw samples of the contents by sterile puncture as desired, and thus to determine the nature of the normal flora in relation to the acidity or alkalinity of the intestinal contents, and also the fate of other bacteria when administered by the mouth.

Table 65 shows the relation between the hydrogen-ion concentration of the intestinal contents and the prevailing bacterial flora at different levels.

TABLE 65

Portion of Small Intestine.	pH of Contents.	Bacterial Flora.
Duodenum	5.2-6.0	Few Gram-positive cocci.
Jejunum, upper half . . .	5.5-6.5	"Gram-positive" cocci, "few Gram-positive and Gram-negative bacilli.
Jejunum, lower half . . .	6.0-7.0	Rich and varied bacterial flora.
Ileum	6.8-8.0	

When watery suspensions of *Chromobacterium prodigiosum* and *Ps. pyocyanea* are put into the empty stomach of a dog 12 to 18 hours after a meal, they fail to reach the cæcum. Introduced in an alkaline buffered watery suspension or in alkaline buffered milk they reach the cæcum in large numbers. (See also Teale 1934 and Chapter 90). Whether the bactericidal action of the duodenal contents is entirely determined by its hydrogen-ion concentration, is, perhaps, open to question (see Meyer and Löwenberg 1928).

Garrod (1937) found that gastric juice was bactericidal for a number of organisms that infect by the mouth. *Br. abortus* was most susceptible; and after it, in diminishing order of susceptibility, *Br. melitensis*, *Salm. typhi*, *Salm. typhi-murium* and *Sh. flexneri*. Gastric juice and washings are also bacteriostatic for *Myco. tuberculosis* (Schwartz 1945, Kramer 1946).

With a view to understanding its significance in defence, Goldsworthy and Florey (1930) studied the distribution of Fleming's lysozyme in mucus and mucosal extracts of the stomach and intestines from several animal species, using as test organisms *Micrococcus lysodeikticus* and a series of unidentified susceptible bacteria isolated from the air of the laboratory. In the cat, no lysozyme could be detected in mucus from the stomach or colon, though small amounts could be demonstrated in saline extracts of the dried mucosæ. In the dog, detectable amounts were present in the mucosa of the intestinal tract, more in the colon than in the stomach. In the rabbit, the mucosal extracts were actively lytic. The colon was more active than the stomach, the small intestine falling between the two. In the guinea-pig, on the other hand, the mucosa from the stomach was more active than that from the intestine. What part this substance plays in eliminating susceptible species from the intestinal flora it is, however, impossible to say.

Quite apart, however, from the action of the contained lysozyme, there can be no doubt that the mucus itself provides a very efficient and important cleansing and protective mechanism. Florey (1933) found that the mucus does not form a uniform coating over the epithelial cells of the mucosa, but is present in the form of a lace-like network spread over the mucosal surface. The villi free themselves from any small adherent particles by movements that bring the particles into contact with the mucus, to which they adhere.

Solution B:	
phenol	5 gm.
distilled water	95 ml.

Mix the two solutions in the above quantities

Ammonium Oxalate Crystal Violet (Hucker).

Solution A	
crystal violet (90% dye content)	2 gm
ethyl alcohol (95%)	20 ml.

Solution B.	
ammonium oxalate	0.8 gm.
distilled water	80 ml

Mix the two solutions in the above quantities

Safranin

safranin (2.5% solution in 95% alcohol) . . .	10 ml
distilled water	100 ml.

Albert's Diphtheria Stain (Laybourn)

toluidine blue	0.15 gm
malachite green	0.02 gm.
acetic acid (glacial)	1 ml.
ethyl alcohol (95%)	2 ml
distilled water	100 ml

Preparation of Smears. Slides should be clean and free from all grease, and may be cleaned in soap and water, thoroughly rinsed, and stored in alcohol or xylol. Before use a slide is passed through the bunsen burner flame two or three times. A drop of distilled water is placed on the cooled slide and a small amount of bacterial growth suspended in it, spread and allowed to dry in air. The film is then fixed by passing through the burner flame two or three times, or, for some stains, by immersion in absolute methyl alcohol, glacial acetic acid or other fixatives.

Procedure of the Simple Stain. The fixed smear is covered with stain, allowed to stand for 30 to 60 seconds, rinsed off with tap water, excess water removed by blotting and allowed to dry in air. Bacteria are usually examined under the oil immersion objective, and the oil can be placed directly on top of the smear.

The Gram Stain. Of the differential stains, the Gram stain is one of the most valuable and most generally applied. The procedure is essentially one of staining with crystal violet, mordanting with iodine solution, decolorizing with alcohol and counterstaining with a dye of contrasting color. The stain was originally developed by the histologist Gram in an effort to stain bacteria in tissues differentially, and has been subject to many modifications. By this procedure bacteria are separated into two groups, those which retain the crystal violet and are said to be *gram-positive*, and those which are decolorized and stain with the counterstain which are *gram-negative*. The distinction is not always sharp for there is considerable variation in ease of decolorization, some gram-positive bacteria such as the pneumococcus become gram negative after they die, and some bacteria are gram variable. This last group is not large enough to detract from the practical value of the stain.

The Mechanism of the Gram Stain. Both the crystal violet and the iodine are highly specific while the alcohol and the counterstain are not. Almost all other dyes, even methyl green which differs by only one methyl group from crystal violet, are unsuitable, either being retained or removed by alcohol whether or not the iodine is applied. A few other reagents, notably

passages with an efficiency of 87.5 per cent. The efficiency was less—62.0 per cent.—when air containing only droplet nuclei was tested. The smaller the particle, and the slower the rate of inspiration, the more readily does it pass the nasal filter (Boyland *et al.* 1947; see also Druett *et al.* 1953, Harper and Morton 1953).

Whether mechanical clearance is the only factor concerned is again uncertain. Arnold, Ostrom and Singer (1928) introduced *Chromobacterium prodigiosum* and *Bact. coli*, by spraying, into the nose of men, rabbits, dogs and guinea-pigs, and noted their rapid disappearance. They state that swabs taken from the posterior pharyngeal wall gave no indication that there was any extensive passage of the bacteria to the throat; but they were unable to detect in nasal washings or secretions any substance that was bactericidal to the test organisms employed. It may be noted that the rate of disappearance recorded by Arnold and his colleagues—a diminution in numbers during the first 5 minutes, and total disappearance within half an hour—was more rapid than that recorded by Bloomfield—little change in 2 hours but almost complete disappearance in 24. This slower rate of elimination was also noted by Bloomfield in the case of particles of kieselguhr placed on the nasal septum. The route followed by substances introduced into the nose to some extent depends on the animal tested, the nature of the substance and the method of its administration. Rake (1937), for example, records a ready intercellular penetration of the olfactory mucosa of mice by pneumococci, *Salm. enteritidis*, particles of Prussian blue and the virus of equine encephalomyelitis, but only slow penetration by rabies and certain other viruses. In rabbits, virulent pneumococci penetrate the nasal mucosa only if the containing fluids are kept in prolonged contact with it (Cannon and Walsh 1937).

Lysozyme is known to be present in high concentration in the nasal secretions, and it is probable that it plays a part in freeing the nasal cavities from those species of bacteria against which it is active. A substance resembling Dold's inhibine in bactericidal power has been described in the nasal secretion of normal persons (Ignatius 1936). Burnet, Lush and Jackson (1939) found a heat-labile, possibly enzymic, substance in nasal secretion that inactivated certain viruses, notably those of influenza, herpes and poliomyelitis. Vaccinia virus was insusceptible to its action. (See also Francis and Brightman 1941.)

Whatever may be the nature of the complex of mechanisms involved there is every reason to suppose that the same result is attained in the nose as in the mouth. The majority of bacteria that enter at the external nares are removed or destroyed, and fail to obtain any but the most transient footing among the normal nasal flora; those that survive and multiply do so because they are in some way adapted for successful colonization, and once such colonization has occurred they may persist for an indefinite period (see also Chapter 90).

The conditions in the nasopharynx have been sufficiently dealt with in considering the elimination of bacteria from the mouth.

It would appear (Calamida and Bartarelli 1902) that the accessory nasal sinuses are normally sterile.

There is little doubt that in health the trachea and bronchi contain few if any living bacteria (see Thomson and Hewlett 1896, Bloomfield 1922*d*); and it is probable that the main protective mechanism consists in the filtering action of the nasal passages combined with the adherence of bacteria which pass the posterior nares, or are drawn in *via* the mouth, to the pharyngeal or upper laryngeal mucosa. It is, however, quite certain, from the ordinary experience of the post-mortem room, that dust particles, and hence presumably bacteria, reach the lungs in numbers that, if minimal in any short period of time, attain a considerable total in the course of months or years.

Under certain conditions, mainly experimental (see Nenninger 1901, Paul 1902,

HgCl₂, may be substituted for iodine, and stannous chloride, pyrogallol and freshly prepared solutions of hydroquinone show some slight activity.

However arbitrary Gram's method of staining may appear, the reaction is apparently associated with fundamental differences between the gram-positive and gram-negative organisms. There is, for example, a pronounced correlation between it and resistance to the bacteriostatic action of certain dyes and antibiotic substances, and to the action of other chemical and physical agents. The nature of the gram reaction has, therefore, been of considerable interest and three general types of theories to explain the differential staining have been developed.

(1) The theory of differential membrane permeability was proposed by Benians,³ whose experimental evidence was consistent with the view that while the cell membrane of both gram-positive and gram-negative bacteria is permeable to the crystal violet and iodine reagents, that of the former is not permeable to the alcohol-soluble dye-iodine complex and hence the color is retained.

(2) The colloidal chemical theory was advanced by Stearn and Stearn⁴ who postulated a relationship between the isoelectric point of the cell protoplasm and its affinity for basic dye on the basis of the negative response of gram positive bacteria at acid pH's, and related evidence; the isoelectric point of gram-positive bacteria is at a lower pH than that of the gram-negative bacteria and therefore the former have a stronger affinity for the basic dye.

(3) A morphological theory was developed principally by Churchman.⁵ His evidence strongly supported the view that the protoplasm of all bacteria is gram-negative and that the gram-positive forms are covered by a sheath or envelope of gram-positive material, i.e., the gram-positive cell consists of a gram-negative "medulla" and a gram-positive "cortex."

More recent work has substantiated this third hypothesis. Henry and Stacy⁶ were able to remove an outer layer from gram-positive bacteria by treatment with bile to leave a gram-negative cell or "cytoskeleton." The bile extract, not in itself gram-positive, contained protein, polysaccharide and the magnesium salt of ribonucleic acid. This material could be deposited on the surface of the extracted cells to render them gram-positive again, but could not be deposited on normally gram-negative bacteria. The ribonucleate appeared to be highly specific in that related compounds or other salts of ribonucleic acid could not be substituted for it. This work has been confirmed and extended by Bartholomew and Umbreit⁷ who converted gram-positive bacteria to gram-negative forms by digestion with ribonuclease and, further, were able to replace the bacterial ribonucleate with magnesium ribonucleate prepared from yeast. There was also evidence that the sulfhydryl groups of the protein were involved in some way in the gram reaction as a whole.

³ Benians *Jour. Path. Bact.*, 1912, 17:199, *ibid.*, 1920, 23:401.

⁴ Stearn and Stearn *Jour. Bact.*, 1924, 9:463, 479.

⁵ Churchman *Jour. Exp. Med.*, 1927, 46:1009, *Jour. Bact.*, 1929, 18:413, also in Jordan and Falk: *Newer Knowledge of Bacteriology and Immunology*. University of Chicago Press, Chicago, 1928.

⁶ Henry and Stacy. *Nature*, 1943, 151:671

⁷ Bartholomew and Umbreit *Jour. Bact.*, 1944, 48:567 This paper includes a critical review of the pertinent literature.

species—resulting from localized glandular secretions, or from differences in the amount of moisture in the skin. Thus, the fluid part of human semen contains an inhibitor of *Staph. aureus*, but not of *Bact. coli* (Rozansky *et al.* 1949).

The urethra in the male and female is normally sterile, or contains in the neighbourhood of the meatus a few staphylococci and diphtheroid bacilli. The mechanical flushing action of the urine, combined perhaps with its slightly acid reaction (pH 6.0), is probably an important factor. Dold and Deck (1941) describe a heat-stable type of inhibine in fresh urine, active against a wide variety of pathogenic species. Both Dold (1947) and Björnesjö (1951) describe inhibitors of *Myco. tuberculosis* in normal urine. Björnesjö's substance is heat-stable, dialysable through cellophan, and is antagonized by serum albumin. Tamm and Horsfall (1952) isolated mucoprotein from urine of molecular weight of about 7 million, highly reactive with influenza, mumps and Newcastle viruses, inhibiting their hæmagglutinating activity; its significance in antiviral defence is not clear.

Urine contains lysozyme, which presumably could prevent the growth of lysozyme-sensitive organisms; a high lysozyme content is found in nephrotic children (Wilson and Hadley 1950).

The vagina has a highly characteristic flora, consisting largely of aciduric bacilli of the Döderlein type (see Küster 1929, Schultheiss 1929). The normal vaginal secretion has a marked bactericidal action on many species of bacteria. Thus Menge (1894) found that *Ps. pyocyanea*, staphylococci and streptococci introduced experimentally into the vagina could not be recovered after 21 to 26 hours (see also p. 1390).

Antibacterial substances have been described in cows' milk, active against cocci and coliform bacilli (see Hobbs 1939, Morris 1943, 1945, Green and Pauli 1943, Wilson and Rosenblum 1952, and pp 194, 2316). It is possible that in man such substances contribute to the defence of the lactating mammary gland against certain infections.

The Efficiency of the Bactericidal or Inhibitory Mechanisms Operating on Body Surfaces.

It is extremely difficult to assess the relative protective value of the factors that we have described above, as compared with the mechanisms that we shall discuss in subsequent chapters. It is obvious that they often fail; but how frequently they succeed we cannot tell.

TABLE 67

SHOWING THE PERCENTAGE MORTALITIES OBSERVED IN GROUPS OF MICE AFTER THE ADMINISTRATION OF VARIOUS DOSES OF A VIRULENT STRAIN OF *Salm. typhi-murium* INTRAPERITONEALLY OR *per os*.

Number of Bacilli administered.	Mortality per cent.	
	Intraperitoneal.	<i>Per os</i> .
1,000,000,000	Not tested	48.75
10,000,000	98.8	28.25
100,000	84.7	22.50
1,000	65.9	15.00
10	28.2	Not tested

The gram reaction, therefore, appears to depend on a surface structure of magnesium ribonucleate combined with protein which is present on gram-positive bacteria but lacking on gram-negative bacteria. This explanation is consistent also with the evidence brought forward by others but interpreted differently; for example, the reaction between the crystal violet and iodine reagents and the ribonucleate-protein complex does not occur at acid reactions, hence the reversal of the gram-positive reaction by lowering pH.

Procedure of the Gram Stain. In addition to crystal violet and safranin staining solutions, Lugol's iodine solution is required as a mordant. Its composition is

iodine	1 gm.
potassium iodide	2 gm.
distilled water	300 ml.

The staining procedure is as follows.

- (1) The heat fixed smear is stained for 1 minute with ammonium oxalate crystal violet.
- (2) Wash in tap water.
- (3) Flood with Lugol's iodine solution and allow to stand 1 minute.
- (4) Wash in tap water and blot dry.
- (5) Decolorize 30 seconds with gentle agitation in 95 per cent alcohol, and blot dry.
- (6) Counterstain 10 to 30 seconds in safranin.
- (7) Wash in tap water, blot dry and examine.

✓ **Acid-Fast Stain (Ziehl-Neelsen Method).** Certain bacteria, characterized by a high lipid content, cannot be stained by the usual procedure of the simple stain and either heat or prolonged contact is required to drive the stain into the cells. Conversely, the stained forms are equally difficult to decolorize and resist decolorization with acid alcohol. These organisms are designated acid fast and include the tubercle bacilli and related forms, the leprosy bacillus and certain of the actinomycetes.

Staining Procedure:

- (1) Stain the smear for 3 to 5 minutes in steaming Ziehl's carbol fuchsin. An alternative procedure useful in diagnostic laboratories is to stain in the cold for 18 to 24 hours.
- (2) Rinse in tap water.
- (3) Decolorize in 95 per cent ethyl alcohol, containing 3 per cent by volume of concentrated hydrochloric acid, until only a suggestion of pink color remains.
- (4) Wash in tap water.
- (5) Counterstain for 30 to 60 seconds with alkaline methylene blue.
- (6) Wash in tap water, dry and examine.

Fontana Stain for Spirochetes. The spirochetes stain very poorly or not at all by the usual simple staining procedure, and are usually stained by a silver impregnation method

add, drop by drop, enough more of the silver nitrate solution to produce a slight cloud which persists after shaking. This solution is stable for some months.

Staining Procedure:

- (1) Steam the heat fixed smear in a solution of 5 per cent tannic acid in 1 per cent phenol for 30 seconds.
- (2) Wash for 30 seconds in running water.
- (3) Flood with ammoniacal silver nitrate solution, heat gently, and allow to stand 20

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Capsule Stain (Hiss's Method). The bacterial capsule does not stain in the simple stain or Gram stain procedures. A number of methods of staining the capsules have been used of which that of Hiss is one of the most simple and effective. The stain is an aqueous solution of basic fuchsin or crystal violet, 0.15 to 0.3 per cent and 0.05 to 0.1 per cent respectively.

Staining Procedure. The bacteria should be grown in ascitic fluid or serum medium for maximum capsule development, and bacteria grown on solid media should be suspended in serum for preparation of the smear. The smear is air dried and fixed by heat.

- (1) Stain with either aqueous basic fuchsin or aqueous crystal violet by heating gently until the stain steams.
- (2) Wash off the stain with 20 per cent aqueous copper sulfate solution.
- (3) Blot dry and examine.

The bacterial cells are deeply stained and the capsules a faint blue or pink, depending upon which stain has been used.

Spore Stain. Like acid-fast bacteria, spores are very difficult to stain and appear as unstained in the usual stained smear. The stain may be driven in by heat, and the Ziehl-Neelsen acid fast staining procedure may be used with the modification that decolorization is less rigorous, i.e., 95 per cent alcohol or absolute acetone should be used instead of acid alcohol.

CULTURE OF BACTERIA

Methods of Obtaining Pure Cultures. When fluid culture media are inoculated with substances such as soil, water or excreta, many kinds of organisms develop simultaneously side by side, and a heterogeneous mixture, or mixed culture, of bacteria results. Any technical procedure for obtaining such pure cultures is dependent upon the isolation of a single viable bacterium which is allowed to multiply in a suitable culture medium. The first reliable method of isolation of pure cultures was devised by Koch in 1881. This method has proved so satisfactory that it has been employed to the present day with only minor modifications. If nutrient gelatin or agar is inoculated while fluid (for example, at 42° C.) and is then solidified and kept under favorable temperature conditions, many of the living bacteria that have been introduced are able to multiply. Since the bacteria cannot move about freely, but are fixed in the stiffened medium, the progeny of each organism form distinct masses or colonies. These colonies consist of many millions of bacteria and are readily visible to the naked eye or by means of a low power hand lens. If the colonies are not closely crowded, a pure culture may be obtained by touching a colony with the tip of a sterile needle and inoculating tubes of fresh culture media. In order to secure a large surface upon which the colonies shall be spread out and made easily accessible, the gelatin or agar after inoculation is poured while still fluid into sterilized flat shallow dishes (petri dishes) fitted with glass covers.

It will be clear that a given colony may arise from two or more parent cells if these remain attached or close together in the agar medium, and the colony will not be a pure culture in the event that the juxtaposed cells are of different species. This possibility has been investigated by McNew* using the plant pathogen *Phytophthora stewartii*. He found that 98 per cent or more of the colonies arose from single cells, hence the probability of a mixed culture in a single colony is 0.02 or less. Successive plating or picking a number of colonies

* McNew. *Phytopathology*, 1938, 28: 387.

to the stimulus provided by the toxin in the infected tissues by a rapid and copious outpouring of antitoxin (the "secondary response"; see Chapter 50); so that the toxin production by the bacterium is more than counterbalanced by the antitoxin production in the host. The 0.01 unit per ml. level is an unreliable index of immunity for two reasons. Firstly, the protective value even of passively administered antitoxin, when there is no question of a later secondary response, varies widely from individual to individual in both man and animals. Thus for a given dose of toxin, the amount of antitoxin required to protect each of an apparently homogeneous group of rabbits may vary over a 100-fold range (Ipsen 1946). Secondly, there is no necessary connection between the serum level and the degree of secondary responsiveness upon which active immunity largely depends. Similar levels might occur in persons whose primary conditioning—for example by a mild infection—was feeble, and in persons with a high degree of responsiveness due to recent immunization (see, *e.g.*, Barr and Glenny 1945, Ipsen 1946). Moreover, the invasiveness and the toxigenicity of the infecting bacilli, and the number in which they arrive at the susceptible tissue, may be such as to overwhelm even a high degree of specific immunity. With these reservations in mind, however, we may regard 0.01 unit per ml. as an average level above which we should attempt to raise the non-immune. The higher it is raised by active immunization, the less likely is infection to result in disease, and the smaller is the probability of complications or death in those who do contract it (Ipsen 1946, Hartley *et al.* 1950.)

Although deviations from this roughly quantitative relation can be attributed mainly to variations in the animals' resistance or the severity of the infective attack, it is possible that differences between one antitoxin and another affect the firmness of toxin-antitoxin union, and so the degree of protection afforded.

We saw in Chapter 7 that antitoxins varied greatly in avidity for toxin; and it is natural to suppose that a relatively feeble combining power would be associated with a low protective power *in vivo*. It should be noted, however, that highly avid sera are usually produced by prolonged immunization of a kind designed to yield high-titre antitoxins; and although it is entirely proper to insist that antitoxins for passive serum treatment of diphtheria should have both qualities, we do not know whether avidity is necessarily an effective quality of the low-titre antitoxins that occur naturally in man.

The increased resistance afforded by specific antitoxic immunity is determined almost entirely by the interception and neutralization of the toxin before it reaches the susceptible cells. Once these have been attacked, the antitoxin is relatively ineffective.

Glenny and Hopkins (1925) studied the effect of various doses of antitoxin at different times after the intradermal injection of a Schick dose of diphtheria toxin into a guinea-pig. This amount of toxin produces a characteristic local reaction at the site of inoculation; 0.001 unit of antitoxin is sufficient to prevent this reaction when the toxin and antitoxin are mixed together before inoculation. When, however, the toxin is injected before the antitoxin, neither 10 units of antitoxin injected intravenously 15 minutes later, nor 1,000 units injected intravenously 30 minutes later, are sufficient to prevent the appearance of a small reaction at the site of the injection of the toxin. The reaction is, however, much smaller than that which occurs when no antitoxin is given. When 10 units of antitoxin are administered intravenously 30 minutes after the intradermal injection of the toxin, or 1,000 units intravenously after 1½ hours, or intramuscularly after 45 minutes, the lesion is reduced to about half its usual diameter. A slight reduction in the size of the reaction is obtained when 1,000 units are injected intravenously after 2½ hours, or intramuscularly

makes a pure culture a practical certainty. It is likely, however, that this figure differs for different bacteria, *i.e.*, staphylococci are more likely to remain attached than micrococci or bacilli.

It is self-evident that the pure culture so obtained is not only homogeneous with respect to kind or species of bacteria, but since the microorganisms are descendants of a single parent cell, they constitute what the zoologist or protozoologist terms a *clone*.

Technique of Making Plate Cultures. Three tubes of agar (1, 2, 3), melted at 100° C., are placed in a water bath at 42° C., a temperature that is just above the solidifying point of agar and is not injurious to bacteria. Tube 1 is inoculated with a loopful of the material to be plated. The cotton plug is then replaced and the contents of the tube are mixed by carefully tilting back and forth and rotating the tube on its long axis. From this tube two loopfuls of agar are transferred to tube 2, and after mixing, two more loopfuls carried from tube 2 to tube 3. The contents of the several tubes are then poured into petri dishes. As soon as the cotton plug is removed, the mouth of each tube should be passed through the flame and inserted under the edge of the lifted petri dish cover, and the agar quickly poured out. The covered petri dish may then be tipped cautiously back and forth to distribute the agar evenly before it solidifies. Agar plates placed in the incubator after solidification should be inverted in order to avoid spreading of the growth through condensation of moisture on the surface of the medium. Even if there are a great many bacteria in the original material, the plate from tube 3 will probably contain the organisms in small enough numbers to develop well isolated colonies. On the other hand, if there are very few bacteria in the material inoculated, plate 1 will probably present more satisfactory conditions. Gelatin plates are made in the same manner as agar except that gelatin may be cooled as low as 25° C. without solidifying.

Under exceptional conditions, such as work in the field, when petri dishes are not available, so-called "roll tubes" may be made. The tubes containing the liquid agar medium are tilted until the agar almost reaches the cotton plug. The tubes are then rotated in this position against a block of ice, and when the process is complete the test tube is coated on the inside with a thin layer of solid medium. In this way a considerable surface is obtained and, after incubation, colonies are readily picked.

Quantitative Dilution. It is sometimes of advantage before plating to make accurate

(A)	To dilute 1	10	use 1 ml. of sample to	9 ml. of sterile water.
(B)	" 1	100	" "	" " " "
(C)	" 1	1,000	" "	(A) " " " "
(D)	" 1	10,000	" "	(B) " " " "
(E)	" 1	100,000	" "	(C) " " " "
(F)	" 1	1,000,000	" "	(D) " " " "

One milliliter of each dilution is placed in a sterile petri dish and 6 to 8 ml. of liquid cooled agar medium are added. The two are thoroughly mixed by tilting the petri dish back and forth and then allowed to solidify. After incubation the total number of colonies on the plates containing 50 to 200 colonies is counted and the total number is multiplied by the dilution to give the number of organisms present in the original sample. Such bacterial counts are relatively inaccurate, errors of 10 to 15 per cent being common in even the most careful work, and must be regarded only as useful approximations.

Streak Plates. Not infrequently media used for the isolation of the more fastidious bacteria are such that it is technically difficult to make the usual pour plates. The most common of these media are those which contain fresh blood. Such media are ordinarily prepared in quantity and sterile plates poured. These may be stored in the ice box until

toxin plays any important part in the invasive process, the antitoxin might to that extent lessen the infection. But we should expect modification of the infection rather than complete immunity. The available evidence is in accord with our expectations.

As regards diphtheria, we discuss in Chapter 61 the evidence for other toxic components of *C. diphtheriae*, which antitoxins as usually prepared fail to neutralize. There are clinical indications that immunity may be referable to bacterial antigens other than the toxin. Thus, nurses in fever hospitals with no detectable antitoxin remain healthy in the face of a continuous risk of diphtheria, suggesting that they may have an *antibacterial* immunity. Lack of such immunity may also explain the not uncommon occurrence of diphtheria in persons with a high antitoxin level; and although in some of these cases the diphtheritic patients may be exhibiting a secondary response to the toxins produced in the early stages of their disease (see Hartley *et al.* 1950), for others this view is not tenable. Indeed, in the light of the recent demonstration of the heterogeneity of purified diphtheria toxin, we are in no position to assert that effective antitoxic sera owe their efficacy to antitoxin alone; or that toxoid is the only protective antigen in diphtheria prophylactics. Toxins from *C. diphtheriae* appear to be homogeneous in that they are all specifically neutralized by a single antitoxin; but strains differ in their somatic antigens (see Hewitt 1948). Frobisher and his colleagues report in rabbits an increase in resistance to toxigenic *C. diphtheriae* which, after immunization with avirulent non-toxigenic strains, was associated with greater tissue reactivity to injected bacilli. No antitoxin was detectable in the partly resistant animals, and the resistance was to some extent associated with serological type-specificity (Frobisher and Updyke 1947, Frobisher and Parsons 1950).

The presence of circulating antitoxin, active against the rash-producing toxin of the hæmolytic streptococcus, affords protection against clinical scarlet fever, that is, against the obvious effects of the toxin itself; but it does not appear to protect against the local lesion in the throat—the acute tonsillitis (Okell 1932). Again, it would seem (Burt-White, Colebrook and others 1930, Baird and Cruickshank 1930, Stent 1930) that the presence of circulating antitoxin, as revealed by a negative Dick test in pregnant women during the later stages of gestation (see p. 1676) affords little if any protection against a severe or fatal infection with hæmolytic streptococci during the puerperium. These clinical findings are borne out by the results of experiment. Thus, Parish and Okell (1927) found that a potent antitoxic serum, which would protect rabbits against acute toxæmic death following the intravenous injection of large doses of living cultures of hæmolytic streptococci, did not prevent a fatal infection, leading to death after a week or more, associated with multiple foci of infection. Nevertheless, as reference to Chapter 66 will show, in patients with scarlet fever, antitoxic sera sometimes appear to influence the course, not only of the toxæmia, but of complications directly referable to the bacterial invasion by the *Str. pyogenes*.

Closely analogous results were obtained in experimental staphylococcal infections in the rabbit (Burnet 1929, Burnet and Kellaway 1930, Kellaway, Burnet and Williams 1930). The potent staphylococcal toxin is neutralized, in constant proportions, by the specific antitoxin; and a rabbit can be actively or passively immunized against the poisonous effect of a toxin-containing filtrate. But such immunized animals are still susceptible to the injection of living staphylococci and succumb to a pyæmic infection, though they survive rather longer than non-immunized controls. Later studies, however, indicate clearly that in rabbits antitoxin protects, though not completely, against bacterial invasion. Ramon and his colleagues (1936*a, b*) found that intravenous virulent *Staph. aureus* did not kill rabbits with a high level of antitoxin in the serum, whereas rabbits with a low level succumbed. Kitching and Farrell (1936) observed that immunization with toxoid protected rabbits against infection, but that immunization with bacterial bodies, though it produced abundant agglutinins, was ineffective. Both Forssman (1936) and Smith (1937) demonstrated that active immunization with staphylococcal products was

There are four general categories which, in the order of the fineness of distinction they make possible, are: (a) morphology, both gross and microscopic; (b) physiologic capabilities in terms of biochemical reactions; (c) pathogenicity for experimental animals; (d) immunologic character.

The preliminary and basic study of a bacterium lies in the systematic determination of its cultural characters. The most important of these are:

(A) Morphology

1. gross morphology—that of colonies of the organism with respect to size, texture, color, shape, etc.
 - (a) on nutrient or infusion semisolid media
 - (b) on special media
2. microscopic morphology, including
 - (a) size, shape and grouping of the organisms
 - (b) presence or absence of spores
 - (c) motility
 - (d) presence or absence of capsule
 - (e) staining reactions

(B) Biochemical reactions

1. the fermentation of sugars, usually dextrose, lactose and sucrose, although others may be included together with the hydrolysis of starch
2. liquefaction of gelatin
3. formation of indol
4. reduction of nitrate to nitrite
5. production of hydrogen sulfide
6. special biochemical tests such as the Voges-Proskauer reaction, the methyl red test, hemolysis on blood agar, etc.

Such preliminary examination ordinarily affords a great deal of information about a given bacterial culture. The feasibility of additional study rests firmly on the foundation laid down by the studies outlined above.

Various attempts have been made to systematize the study of bacteria. One of the most successful of these has resulted from the efforts of a Committee on Bacteriological Technique of the Society of American Bacteriologists, under whose auspices a Manual of Pure Culture Study has been published and is supplemented by leaflets from time to time. Through this agency standard methods and descriptive terminology are made available. Furthermore, the same committee has prepared a standard descriptive chart for the recording of data, and from time to time the chart is revised.

ANIMAL INOCULATION

An indispensable adjunct to the study of the pathogenic bacteria is the experimental animal. Animals are used not only for the study of the pathology of infectious disease and as an aid in the isolation of some bacteria in pure culture, but also for the experimental production of immune sera and studies on the various manifestations of immune phenomena. The maintenance of such infectious agents as the filterable viruses, which cannot be cultivated on lifeless media, by animal passage is common also. The animals generally used are rabbits, guinea pigs, white mice and white rats, although in special cases others, such as rhesus monkeys, are necessary.

Routes of Inoculation. Experimental animals may be inoculated by a variety of routes, usually one or another being preferable under the particular cir-

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Name of organism Culture No.

Date of isolation Temp. °C.

Is phase variation observed? Parts:

Underscore required terms.

VEGETATIVE CELLS: Medium used CHARACTERIZATION

Reaction (pH) Temp. characteristics is determined, indicate in proper marginal

..... designated below. In case any of these characteristics are

..... determined, indicate with the letters U, V, and X according

..... determined; V, variable; X, doubtful.

..... 3, micrococci;

..... 0, filamentous

IRREGULAR FORMS: 1, positive

Present on in days nitric, 2, polar; 3, present but undetermined

..... Temperature 1, positive

..... entire, undulate, lobate, erose, 2, dull

..... photosynthetic, yellow, 2, contoured, 3, rugose

Lustrous, glistening, dull, 1, pathogenic for man; 2, for animals but not for man;

Chromogens: 3, but not pathogenic; 4, saprophytic; 5, autotrophic

Odor, absent, decided, resembling 1, strict aerobe; 2, facultative anaerobe; 3, strict

Consistency, butyrous, viscid, membranous 1, nitrate nor gas; 1, both nitrate and gas; 2, nitrate but

Medium, grayed, browned, reddened, black, 1, nitrate

Temperature 1, pink; 2, violet; 3, blue; 4, green; 5, yellow;

NUTRIENT BROTH: 1, white, 0, black

Surface growth, ring, pellicle, flocculent, wet 1, none; 1, photogenic, 2, fluorescent, 3, iridescent

Clouding, slight, moderate, strong, transient, 1, none

Sediment, turbid, granular growth, 1, none

Odor, absent, decided, resembling 1, none

Sediment, compact, flocculent, granular, flaky 1, none

Amount of sediment, abundant, scanty, none 1, none

GELATIN STAB: Temperature 1, positive

Growth, uniform, best at top, best at bottom, 1, positive

Line of puncture, diffuse, beaded, papillate, 1, positive

Liquefaction, none, crateriform, indefinite, 1, positive

..... 1, positive

Degree of liquefaction in 1, positive

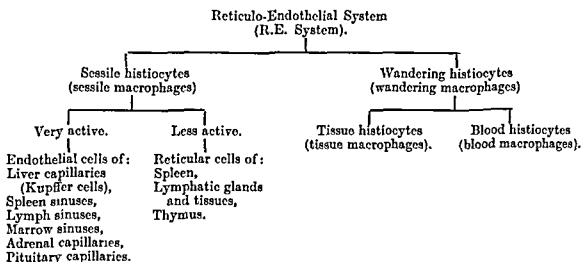
Method used 1, positive

Medium, fluorescent, browned, unchanged, 1, positive

Medium	Arabinose	Rhamnose	Xylose	Monosaccharide
containing				1, positive
				1, negative; 1, positive
				1, negative; 1, positive
				1, positive
and:				1, negative; 1, positive
Gas in				1, positive
fermentation tube				1, positive
And CO ₂ in Eldredge tube				1, positive
Reaction (pH) after				1, positive
Turbidity in ml. of				1, positive
1% NaOH				1, positive

most active of the sessile histiocytes are those found in certain specialized regions of the vascular or lymphatic endothelium—the liver capillaries (Kupffer cells), the spleen sinuses, the venous sinusoids of the bone-marrow, the capillaries and medullary sinusoids of the adrenals, the capillaries of the pituitary gland, and the sinuses of the lymph glands throughout the body. Somewhat less active in this respect are the reticuloocytes, which are not part of the lining to blood or lymphatic channels, but are disposed about the reticulum fibres in the interstices of the tissues. The wandering histiocytes are found throughout the tissue spaces, and some of them find their way into the circulating blood, particularly into the vessels of the internal organs.

The common character of dye-storage possessed by cells of this type led many observers, and particularly Aschoff (1924), to regard them as an integrated system of cells fulfilling a particular bodily function. Aschoff coined for them the name *reticulo-endothelial system* (R. E. system). The cells of this system may be classified as follows :



The schema classifies the various cells belonging to the system, and does not necessarily represent a genealogy of the cells found in the animal body. The interrelations of the cells in this schema are far from clear; but subsequent work has indicated some which are relevant to our discussion. The R. E. system is part of a larger system of connective tissue cells that participate in defence, and the reactions to infection consist not only of phagocytosis and digestion of the invading organism, but of development and proliferation of phagocytes from other mesenchyme cells. Many of the cells in the R.E. system have this developmental potentiality, but to these must be added cells not in the system, especially the non-phagocytic lymphocytes and plasma cells which may also give rise to macrophages (Rebuck 1947). Taliaferro and Mulligan, indeed, substitute for the R.E. system the *lymphoid-macrophage system*, which contains all the potential and actual macrophage cells, including the non-granular leucocytes (see Taliaferro 1949). What we have to say in the following pages about the R.E. system is in general valid for the wider lymphoid-macrophage system. Both exclude, and are in contrast with, Metchnikoff's *microphage* connective tissue cells, namely the granular polymorphonuclear leucocytes. For our purpose the R. E. system is a functional unit consisting of cells derived from the mesenchyme, having in common a phagocytic activity (see, for example, Doan 1940). We are particularly concerned with



The Reactions which follow the Intravenous Injection of Bacteria into Normal Animals.

There is no doubt at all that the mechanism brought into play in clearing the blood stream from bacterial cells is in the main identical with that which frees it from inert particles of the same order of size (Wyssokowitch 1886, Werigo 1894, Opitz 1898, Bardach 1889, Métin 1900, Bail 1905, Bull 1914-16, Kyes 1916, Bartlett and Ozaki 1917, 1918, Wright 1927 and many others). The reticulo-endothelial cells, particularly those of the liver, spleen and bone-marrow, actively phagocytose the injected bacteria. The mechanism of aggregation, followed by the removal of the bacterial aggregates from the general circulation by their retention in the lung capillaries, is sometimes very prominent (Bull 1914-16, Bartlett and Ozaki 1917, 1918, Wright 1927, Dudgeon and Goadby 1931). This is accompanied, as in the case of the intravenous injection of carbon particles, by an accumulation of polymorphonuclear cells in the lung capillaries, associated with a temporary peripheral leucopenia (see Levaditi 1901, Andrewes 1910). But the polymorphonuclear cells appear to play a more active part in phagocytosing bacteria than in phagocytosing carbon particles (Dudgeon and Goadby 1931). It is probable that these cells with their ingested bacteria are subsequently carried to the reticulo-endothelial depôts in the spleen, liver and elsewhere, and are there phagocytosed by the sessile histiocytes. Such wandering histiocytes as are present in the lung capillaries ingest the bacterial cells, or cell-aggregates, directly.

So far then as we can regard bacteria merely as foreign particles, our description of the reaction of the tissues to the intravenous injection of vital stains or carbon particles needs little modification. But, in fact, the bacteria we inject are alive and capable of multiplication; and it is the balance between their capacity to multiply and the capacity of the tissues to remove them that determines the fate of the animal host.

Bull (1914-16) counted the viable pneumococci and streptococci in the systemic blood stream of dogs and rabbits at various intervals after the intravenous inoculation of a bacterial suspension. There was at first a sharp and progressive fall in the number of organisms in the circulating blood, lasting until about the 5th hour; then a secondary rise, varying in degree and persistence with the virulence of the organism and the resistance of the host. With strains of low virulence the numbers then declined until the blood became sterile. With typhoid bacilli the fall in numbers was very rapid, from 10,000,000 per ml 1 minute after injection to 40 per ml. at the end of 15 minutes.

Wright (1927) made a detailed study of pneumococcal septicæmia in the rabbit, and the following examples are taken from his paper.

TABLE 68

LIVING PNEUMOCOCCI PER ML. OF CIRCULATING BLOOD AT STATED TIMES AFTER INOCULATION OF AVIRULENT, SLIGHTLY VIRULENT, AND HIGHLY VIRULENT PNEUMOCOCCI INTO NORMAL RABBITS.

Time	Avirulent.	Slightly Virulent.	Highly Virulent.
Immediately	8,900,000	1,030,000	1,070,000
2 hours	206	20,800	137,000
5 "	2	340	25,000
24 "	0	1,300	1,510,000
48 "	—	134	Dead
96 "	—	0	—

es. The common routes of inoculation are intradermal, subcutaneous, intracuticular, intraperitoneal and intravenous. Other routes such as intracerebral, intrathecal, etc., may be desirable at times. In any case, inoculation is carried out with a syringe and hypodermic needle, the capacity of the syringe and size of the needle used depending upon the quantity of material to be injected and the route of inoculation. The site of inoculation should be prepared by removal of the hair by shaving or a depilatory, followed by disinfection by swabbing with alcohol, tincture of iodine, etc. The syringe and needles have, of course, been previously sterilized by dry heat or by boiling.

Intracutaneous Inoculation. Material inoculated intracutaneously is inoculated into the dermis. A fold of skin is pinched up and the needle inserted, lumen up, as superficially as possible. A raised white spot showing the pits of the hair follicles indicates a successful inoculation. Not more than 0.2 ml. may be inoculated by this route.

Subcutaneous Inoculation. Subcutaneous inoculation requires considerably less skill than the part of the operator. A fold of skin is pinched up as before and the needle inserted through the skin to its full length. The injected material forms a bleb or blister. The amount that may be injected by this route depends upon the size of the animal.

Intramuscular Inoculation. In the intramuscular inoculation, the needle is inserted deep into the muscular tissue, usually the posterior muscles of the thigh or the lateral thoracic or abdominal muscles.

Intraperitoneal Inoculation. Intraperitoneal inoculation is carried out as one would a subcutaneous injection on the abdomen except that after the needle penetrates the skin, it is held at right angles to the peritoneal wall and thrust through into the peritoneal cavity.

Intravenous Inoculation. Intravenous inoculation may be carried out in a variety of ways, the choice depending upon the size of the animal. Rabbits are conveniently inoculated in the marginal vein of the ear. The needle is inserted through the skin and into the vein from the side. If the needle is within the vein, blood will disappear in the vein from the point of the needle onward as the material is injected. Needless to say, the injection should take place in the direction of blood flow. Guinea pigs may be inoculated into the large superficial vein on the dorsal and inner side of the hind leg or into one of the external jugular veins. Both require anesthesia of the animal and a small incision, which may be later closed with a suture or collodion. Rats may be inoculated in an external jugular vein and mice in one of the lateral veins of the tail.

between the ribs at the point of maximum pulsation and thrust into the heart, taking care to injure the pericardium as little as possible. Blood is most conveniently taken from smaller animals by this means.

Postmortem Examination. The autopsy of experimental animals dead of infection is often highly desirable. Not only may cultures be taken, but the gross and microscopic pathology is usually of significance. The animal is fastened down on a board, ventral surface up. It is good practice to have the board in a larger enameled tray to reduce to a

or other changes, and the hair and skin are disinfected with cresol or lysol.

The skin is incised from the pubis to the neck and cut at right angles at the ends of the

bacterial growth (see Chapter 4). Wright showed that this initial clearance of virulent pneumococci can be almost eliminated by injecting a culture that is still in the logarithmic phase.

In the argument from analogy, which plays so large a part in the construction of our concepts of infection and resistance, we must be very careful to allow for the effects of our experimental technique. The reactions which we have described above are those that follow the sudden introduction into the blood stream of a relatively enormous number of bacteria—a number of the order of 1,000,000,000. Such an occurrence must be of extreme rarity in natural infection, and it is possible that some part of the mechanism we have described—for instance, the aggregation of bacterial cells and the retention of the aggregates in the lung capillaries—may have been over-emphasized by the particular experimental procedure that we have chosen to employ. In any case there seems little doubt that the most significant happenings in the primary bacteræmia are those that occur between the 5th and 24th hours after an intravenous inoculation, when the fate of the host is being determined by the balance between bacterial multiplication, on the one hand, and the removal of the newly produced bacterial cells on the other.

The increasing bacteræmia does not appear to be due to any exhaustion of the clearing mechanism. Martin and Kerby (1950) found that the removal of a strain of *Staph. aureus* from the blood of a rabbit suffering from an overwhelming and ultimately fatal pneumococcal bacteræmia was as efficient as that in a normal rabbit. There was no evidence that, in the overwhelming infection, the pneumococci had acquired a resistance to clearance from the blood stream.

Even when the tide has for the moment turned decisively in favour of the host, it does not follow that there will be a complete sterilization of the tissues. Bacteria will have been caught up in the liver, spleen and elsewhere, and may remain alive, but relatively inactive, for considerable periods. For example, in experimental infections of various rodents viable *Past. pestis* and bacilli of tularæmia may persist for several days in the tissues of animals that eventually recover (Meyer *et al.* 1948, Downs *et al.* 1949). In more chronic infections, like tuberculosis, the bacteria persist for much longer periods (see Brieger 1949).

Our knowledge of the exact method by which bacteria are killed in the tissues is woefully incomplete. There is no doubt at all that the majority of the bacteria taken up by phagocytic cells are destroyed by intracellular digestion; but the tacit assumption that a bacterium phagocytosed is, of necessity, a bacterium finally disposed of is certainly unwarranted.

It should be noted that clearance may be effected by the mediation of granulocytes. Braude (1951) for example, recorded the accumulation of polymorphonuclear cells containing brucellæ in the sinusoids of the liver, as well as phagocytosis by the sessile Kupffer cells, within 3 hours of intravenous injection of the organisms into mice; within 24 hours, the granulocytes were largely replaced by macrophages.

The Reactions that follow the Intraperitoneal Injection of Bacteria or of Suspensions of Inert Particles into Normal Animals.

The small quantity of fluid present in the normal peritoneal cavity, or other serous sac, contains very few cells, and these consist almost entirely of mononuclears, mainly of the lymphocyte type (Dudgeon and Ross 1906, Cappell 1930, and many others).

Durham (1897), in his classical paper on the reaction to intraperitoneal infection,

incision. The two flaps may be laid back by separation of the cutaneous from the underlying muscular tissue with a scalpel. The condition of the subcutaneous tissue and of axillary and inguinal lymph glands may be noted. The peritoneal fluid may be cultured by searing a small area on the surface of the abdomen with a hot spatula and puncturing with a syringe and needle or a Pasteur pipette into which the fluid may be aspirated. The abdominal cavity is opened with fresh instruments and the flaps are laid back as above. The condition of the abdominal organs, such as spleen and liver, may be noted and cultures taken by first searing the surface and then either puncturing and aspirating fluid with a syringe and needle or a Pasteur pipette, or by removing a small piece of tissue and placing it in nutrient medium. Likewise, pieces of tissue may be removed and placed in fixing fluid for subsequent sectioning. Again with fresh instruments, the costal cartilages are cut to make a V-shaped incision and this flap is laid back over the head, exposing the thoracic cavity. Any gross pathology may be noted, cultures may be taken from heart's blood and elsewhere if desirable, and pieces of tissue removed for fixation and sectioning. The animal is disposed of by incineration.

It is obviously impossible to define postmortem procedure rigorously. The lymphatic

The routine of examination will be determined, therefore, by the particular disease under consideration or, if this is not known, by the symptoms exhibited by the animal before death.

A number of precautions must be observed in the autopsy of animals dead of infectious disease. If cultures are to be taken, fresh sterile instruments must be used at each stage. Instruments once used should be returned to the boiling water sterilizer or placed in a tray containing lysol or, if to be used again, laid down on the edge of the tray with all sharp edges and points inward. Extreme care must be taken to avoid disseminating infectious

IMMUNOLOGICAL METHODS

The immunological or serological reactions serve to differentiate bacteria on the basis of their constituent antigens. Such immunological differences are usually, though not always, subordinate to the biochemical differentiation of species and in general serve to subdivide species into immunological types or varieties. The immunological identification of an unknown microorganism is of considerable diagnostic utility, and is frequently of very great value in epidemiological studies in tracing the source and dissemination of infection. Furthermore, serum may be tested against a known antigen to establish the presence of the homologous antibody and thus by inference the presence, immediate or past, of the microorganism or its products in the host, and therefore serves as an indirect diagnostic procedure. The specific neutralization of toxin by antitoxin *in vivo* serves to measure antibody in serum or the circulating antibody in the living host, and in the latter is a measure of antitoxic immunity. Of the *in vitro* immunological reactions, those of agglutination of a bacterial or other discrete antigen, precipitation of a soluble antigen, and complement fixation in the presence of soluble or discrete antigen are of general utility.

Titration of Diphtheria Antitoxin by the Römer Method. The intradermal inoculation of diphtheria toxin in the rabbit produces a local erythema, and central necrosis with doses, which may be used in the assay of toxin, and of toxin in the presence of antitoxin. The end point ordinarily taken is the smallest amount of toxin that, in a dose of 0.2

carrying with it, as in a retracted net, the particles caught on its surface layers. The mechanism of these movements was studied by Florey and Carleton (1926). They found that the omentum is totally incapable of any intrinsic movement. The movements that it undergoes are impressed on it by (a) posture, (b) the peristaltic movements of the intestines, and (c) the movements of the diaphragm. The retraction into a tightly gathered transverse band is due to the alteration in the character of the omental surface, and particularly to the deposition of fibrin, which causes the omental folds that are brought into apposition to adhere to one another, instead of extending again in response to the next passive movement.

The reaction that follows an intraperitoneal injection is not, however, limited to the peritoneal cavity. Indeed, it spreads beyond it with surprising rapidity.

Muscattello (1895) injected carmine suspensions into the peritoneum, killed the inoculated animals after various intervals, and studied the distribution of the dye. Within 1-2 hours he found particles of carmine in the liver and spleen. Similar particles, in smaller numbers, were found in the lungs, pancreas and testis. Durham (1897) noted the rapid passage of bacteria from the peritoneal cavity to the blood stream, and concluded that the most important route of transit was via the diaphragmatic lymphatics, the anterior mediastinal glands and the right lymphatic duct. The importance of the diaphragmatic route was also noted by Dudgeon and Ross, and the correctness of Durham's conclusions has been confirmed by many subsequent observers, notably in the careful studies of Buxton and his co-workers, and in the subsequent studies of Bolton (1921). Buxton and Torrey (1906b) found that, almost immediately after the intraperitoneal injection of an Indian-ink suspension, there was a rush of carbon particles into and through the lymphatics of the diaphragm. Thence they passed rapidly through the anterior mediastinal lymphatics and the corresponding lymph glands, reaching the blood stream within a very few minutes. During the earliest stages of this transit there was very little evidence of phagocytosis in the mediastinal glands or elsewhere; but, as time passed, an increasing number of carbon particles were found within the histiocytes of the lymph nodes.

In other experiments (Buxton 1906, Buxton and Torrey 1906a) the fate of living typhoid bacilli, injected intraperitoneally into rabbits, was followed by quantitative plating methods. The living bacilli passed into the general circulation almost immediately; and their numbers per ml. of circulating blood reached a maximum after about an hour. After this there was a rapid decrease, and few bacilli could be recovered from the blood after about the 6th hour. When suspensions of liver and spleen tissue were plated it was found that there was a rapid accumulation of bacilli in these organs, and particularly in the liver, within a few minutes after an intraperitoneal injection. There was then a transitory decrease in the numbers of viable organisms, followed by a secondary increase lasting from the 2nd to the 6th hour, i.e., the bacilli were increasing in the liver and the spleen while they were decreasing in the blood. After about the 6th hour there was a general decrease, lasting for several days, and more rapid in the liver than in the spleen. When animals were killed 4 days after injection viable bacilli were still recovered from the liver, though in small numbers; larger numbers were recovered from the spleen, and very large numbers from the mediastinal glands. Many observations on laboratory animals have shown that living bacilli may be recovered from the spleen weeks or months after experimental infection (Topley and Wilson 1923, Price-Jones 1927).

It may be noted that the rapid transit of particles or bacteria from the peritoneum to the blood stream is almost certainly the result of the mechanical pumping action of the rise and fall of the diaphragm in breathing. This is probably assisted by a special arrangement of the endothelial cells of the peritoneal membrane covering the diaphragm, which provides for a rapid passage of particles to the underlying lymphatics (Florey 1927; see also Bangham *et al* 1953)

ml, will produce a zone of erythema 10 mm. in diameter 48 hours after inoculation. This is designated the minimal reacting dose or MRD, and the activity of a toxin may be measured as MRD per ml.

Such a titration measures the potency or toxicity of the toxin preparation, but not its combining power with respect to antitoxin. An expression of the activity of the toxin in the presence of antitoxin is the definition of the Lr dose. This is that amount of toxin which, when mixed with 1 unit of standard antitoxin, contains 1 MRD of unneutralized toxin. It will be clear that a toxin so standardized with respect to standard antitoxin can then be used to titrate an unknown antitoxin by using a constant amount of toxin mixed with serial dilutions of the unknown antitoxin, and testing of the mixtures for activity.

One application of this technique is the titration of the antitoxin content of the serum of persons, immunized, convalescent or normal. This will be considered here since it illustrates all of the principles involved. In studies on human immunity the usual procedure is to titrate the serum against an amount of toxin which will just give the end point reaction when mixed with 0.002 units of standard antitoxin, this is commonly spoken of as titration at the 1/500th level.

Standardization of Toxin at the 1/500th Level. Toxins vary widely in potency and combining power and no precise quantitative procedure may be given. The general model of the titration is as follows:

- (1) Assume that a toxin whose L₊ dose is known is available. Then the following preliminary titrations can be carried out:
 - (a) The standard antitoxin is diluted so that it contains 1 unit per ml.
 - (b) The toxin is diluted so that it contains 1 L₊ dose per ml.
 - (c) The toxin is titrated in dilutions of 1:100, 1:200, 1:300, up to 1:1000 against constant amounts of antitoxin, viz., 1 ml. of diluted antitoxin is mixed with 1 ml. of each toxin dilution, incubated as indicated below, 0.2 ml. of each inoculated intradermally in the rabbit, and the reaction read in 48 hours. This titration determines the Lr dose of toxin.
 - (d) Additional preliminary titration indicates the approximate amount of toxin required to just give the skin reaction in the presence of 1/500 unit of antitoxin. A more precise determination is carried out as follows:
 - (i) Prepare a dilution of the original toxin in sterile broth to contain exactly 100 Lr per ml. This is the so-called stock solution and is relatively stable if prepared from stabilized (aged) toxin.
 - (ii) Dilute 1 ml. of the stock solution of toxin with 99 ml. of buffer solution to give a solution containing 1 Lr per ml.
 - (iii) Prepare serial dilutions at intervals of 5 within the limits indicated by the above preliminary titration. Thus, if the end point is indicated as lying between 1:40 and 1:80, prepare 1:40, 1:45, 1:50, up to 1:80.
 - (iv) Mix 1 ml. of each dilution of toxin with 1 ml. of standard antitoxin diluted to contain 0.002 unit per ml. Incubate the mixtures for 1 hour at 37° C. and leave in the refrigerator overnight to allow ample time for the toxin-antitoxin reaction.
 - (v) Inject 0.2 ml. of each dilution mixture intradermally into the shaven skin of a rabbit (as many as 40 such inoculations may be made in a single animal without ill effects) and identify the sites of inoculation with dye.
 - (vi) Read at 48 hours, the end point being that dilution of toxin which produces a zone of erythema nearest to 10 mm. in diameter.

Titration of Antitoxin at the 1/500th Level. For the titration of antitoxin in serum proceed as follows:

- (1) Dilute the stock solution of toxin as indicated in the above standardization, i.e., so that 1 ml. contains sufficient toxin to give the skin reaction when mixed with 1 ml. of antitoxin containing 0.002 unit per ml. and inoculated in amounts of 0.2 ml.
- (2) Prepare dilutions of patient's serum, say, for illustrative purposes, of 1:10 and 1:500.
- (3) Mix 1 ml. of undiluted serum and 1 ml. of each of the dilutions with 1 ml. each of the diluted toxin, and in addition set up a control which contains 1 ml. of the diluted toxin and 1 ml. of standard antitoxin diluted to contain 0.002 unit.

into play, but of the predilection of the bacterium for the tissues it is invading. Very little is known of the peculiarities, chemical or physical, of the bacterial surface that determines its affinities for a given cell or tissue, though several attempts have been made to relate virulence to qualities like surface charge (see Falk and Jacobson 1925, 1926, Jacobson and Falk 1927). Some progress has been made in recent years with the study of the special affinities that viruses have for sticking to certain types of cells (see Chapter 55); a similar study of bacteria might equally illuminate the pathogenesis of bacterial disease.

The Permeability of Blood and Lymph Capillaries to Foreign Matter.—Underlying the various epithelial surfaces of the body there is an extensive anastomotic network of blood capillaries and lymphatic channels. The two networks, with rare exceptions, constitute independent systems, each lined by a continuous sheet of endothelial cells. The lymphatic network drains into the afferent lymphatic channels, which in turn lead to a lymph node (Drinker and Yoffey 1941). In normal tissues, excepting the liver, the permeability of both capillary and lymphatic endothelium is poor. Substances of low molecular weight only are absorbed from the tissues by the capillaries. For instance, Rous and his colleagues (Rous, Gilding and Smith 1930, Smith and Rous 1931*a, b*, Rous and Smith 1931) showed that certain dyes passed directly into the venous portion of a capillary loop. Cobra venom, of molecular weight 5,000, is absorbed directly into the blood stream, but diphtheria and tetanus toxins, of molecular weights greater than 50,000, are not (Barnes and Trueta 1941). Serum proteins do not pass directly into the blood stream from the tissues (Starling 1895-96). The permeability of the blood capillaries increases greatly with trauma, and under the influence of various noxious substances. It does not appear that the passage into the blood stream of particles of bacterial dimensions is facilitated in these circumstances; though Tuttle and Cannon (1935) obtained some evidence that virulent streptococci passed directly into the blood stream after their introduction into the lung alveoli of dogs. The lymphatics, on the other hand, readily become permeable to large molecules and small particles introduced directly into the intercellular tissue spaces, whether they are, for example, inert particles, red blood cells or bacterial in nature (see, for instance, Cappell 1929, Batchelder, Field and Drinker 1931, McMaster and Hudack 1934, Field and Drinker 1936, Schulz, Warren and Drinker 1938, Barnes and Trueta 1941). We have already noted the speed with which bacteria reach the thoracic duct *via* the lymphatics, from the peritoneal cavity, etc. These facts suggest that in areas which, like the skin and subepithelial tissues of the respiratory and alimentary tracts, are liberally supplied with lymphatics, substantial numbers of the invading bacteria are rapidly carried to the lymph nodes draining the site of invasion.

The Filtering Action of Lymph Nodes.—As Drinker, Field and Ward (1934) showed, hæmolytic streptococci or particles of Indian ink introduced into an afferent lymph channel are to a large extent removed from the lymph during its passage through a lymph node. The filtration appears to depend mainly on the complex ramifications of the channels in the node, and to a lesser extent on the removal of particles by phagocytic cells lining the sinusoids of the node. The filtration, however, is by no means complete. Schulz, Warren and Drinker (1938) instilled virulent Type III pneumococci into the nose of rabbits, and recovered cocci from the vessels draining the superior deep cervical node within one hour of the instillation; non-virulent organisms, on the other hand, did not appear in the efferent lymph. Where lymph has to traverse several nodes in its passage to the thoracic duct filtration may be very efficient. Thus Drinker, Field and Ward (1934)

(4) Incubate, titrate, and read as above.

Suppose the t₅₀ mixed with the 1:500 and 1:10 dilutions of serum gives a skin reaction, but that mixed with the undiluted serum does not. It follows that the undiluted serum contains more than 1/500 unit of antitoxin per ml. but it contains less than 1/50 unit per ml. since it is not neutralized by the 1:10 dilution of serum. By varying the serum dilutions, any fraction of a unit may be determined as indicated in the accompanying table.

DILUTIONS OF PATIENT'S SERUM FOR TITRATION OF ANTITOXIN AT 1/500TH LEVEL.

Patient's Serum (ml.)	Saline (ml.)	Dilution	Amount Mixed with 1 ml. of Toxin Dilution (ml.)	Fraction of Antitoxin Unit Tested for
1.0	0	0	1	1/500
0.5	0.5	1 in 2	1	1/250
0.2	0.8	1 in 5	1	1/100
0.1	0.9	1 in 10	1	1/50
0.1	1.6	1 in 16.6	1	1/30
0.1	2.4	1 in 25	1	1/20
0.1	4.9	1 in 50	1	1/10
0.1	9.9	1 in 100	1	1/5
0.1	49.9	1 in 500	1	1/1

The Precipitin Reaction. The precipitin reaction is the specific formation of a precipitate when a soluble antigen and its homologous antiserum are mixed. It is used for the identification of soluble antigens of bacterial extracts, such as pneumococcus polysaccharide, the polysaccharide and protein antigens of the streptococci, etc., and is used for the immunological typing of a number of kinds of bacteria. It is also used for the identification of other protein antigens as, for instance, the identification of bloodstains in forensic medicine.

In the precipitin test antiserum is used undiluted, or in very low dilution which avoids undesirable cross reactions, and is admixed with successive dilutions of the antigen. The titer of the antiserum is expressed as the highest dilution of antigen with which precipitation occurs. It is one of the most delicate and sensitive of the serological reactions, antisera which will precipitate an antigen such as pneumococcus polysaccharide in dilutions as low as 1:100,000 are frequently used.

For the Wassermann test, 10 test tubes, each of which contains 0.5 ml. of saline solution.

- (b) To the first tube add 0.5 ml. of the antigen solution, mix well by blowing in and out of the pipette several times, and transfer 0.5 ml. of this dilution to the second tube. The procedure is repeated until the expected titer, of the antigen, is reached. If the original antigen dilution is 1:2, 1:4, 1:8, etc., but it is more convenient to prepare a 1:100 dilution of the original antigen to begin with, thus giving dilutions of 1:100, 1:200, 1:400, up to 1:25,600, etc.

(2) **Procedure of the Test**

- (a) Distribute the antiserum in amounts of 0.1 ml. in a series of 5 x 50 mm. test tubes, as many as the dilution range to be observed requires.

the capillary walls made permeable by leukotaxine come phagocytes, and an exudate containing antibodies, other antibacterial substances and fibrinogen. The coagulation of the fibrinogen in this exudate leads to the deposition of a fibrin meshwork in the intercellular spaces of the injured tissues. The protein content of lymph will rise, since under the influence of leukotaxine the lymphatic endothelium will have become permeable to the exuded proteins, and under the toxic action of the invading bacteria the lymph will clot in the lymphatic channels. In favourable circumstances, therefore, the intercellular dissemination of the infective material will be hindered by the fibrin deposit in the tissues, and dissemination along the lymph channels by the intralymphatic clots. This clotting process constitutes Menkin's "lymphatic blockade" whereby irritant material is fixed and held at the site of inflammation. If the irritant is sufficiently strong, "fixation" may even precede the diapedesis of leucocytes. According to Menkin, bacteria like *Staph. aureus* stimulate the early formation of a lymphatic blockade, and hence tend to be confined in local abscesses; *Str. pyogenes*, on the other hand, induces a late fixation reaction, and is conspicuously invasive.

The presence of leukotaxine-like polypeptides in the breakdown products of tissue proteins has been demonstrated by Duthie and Chain (1939), and many of the experimental phenomena upon which Menkin bases his hypothesis of inflammatory fixation have been reproduced by other workers, though neither Steinberg and Dietz (1938) nor Lurie (1939) could find any constant association between pH and the predominating type of phagocyte in inflammatory processes. It is probable that a number of factors, including the type of parasite, determine the cellular response, even in the early stages of infective inflammation (see, for example, Gins, Kroemer and Link 1938).

We are chiefly concerned here with the hypothesis of the lymphatic blockade as a defence against bacterial invasion. The chief objection to the hypothesis is the difficulty of demonstrating fibrin deposits and lymphatic thrombi at the periphery of inflamed regions, that is, in the region where they are likely to be effective as a barrier against the spread of infection. Rich (1936) points out that acute inflammatory processes induce an increased, not a decreased, flow of lymph from the injured part (see Field, Drinker and White 1932, Hudack and McMaster 1933), and suggests that foreign substances, including bacteria, are "fixed" in inflamed areas by adsorption to proteins, and by precipitation or other physico-chemical change in the lesion (see also Blalock and Burwell 1935-36); and the work of Miller (1938) on the adsorption of a variety of substances, including bacterial suspensions, from regions of inflammation in rabbits, makes it clear that absorption depends largely on the nature of the substance absorbed. Miles and Miles (1943) attribute the apparent fixation of substances in the inflammatory lesion to an accumulation of exudate in the tissues to such a degree that the existing lymphatic system can drain away only a small fraction during the period of experimental observation, with the result that any foreign material diluted in the exudate appears also to pass slowly from the lesion. Local deposits of fibrin may perhaps improve antibacterial defences in another way. M. R. Smith and Wood (1949) observed that little phagocytosis of pneumococci by leucocytes occurred in citrated rat plasma, but when fibrin strands were produced in the mixture by addition of thrombin, the leucocytes could move on the solid surface of the strands, and pin the cocci against them as a preliminary to a highly effective phagocytosis.

We should at this point emphasize that the term "fixation" is also used for two phenomena that are distinct from the fixation discussed above. The first concerns the

PROTOCOL FOR THE PRECIPITIN TITRATION

Tube Number	Antiserum (ml)	Antigen	
		Dilution	Amount (ml)
1	0.1	1:100	0.1
2	0.1	1:200	0.1
3	0.1	1:400	0.1
4	0.1	1:800	0.1
5	0.1	1:1600	0.1
6	0.1	1:3200	0.1
7	0.1	1:6400	0.1
8	0.1	1:12,800	0.1
9	0.1	1:25,600	0.1
antiserum control	0.1	saline solution	0.1
antigen control	saline solution 0.1	undiluted antigen	0.1

- Carefully layer 0.1 ml. of the antigen dilutions onto the antiserum, each of the serial dilutions in successive tubes, so that the juncture between the two is clear and sharp.
- Incubate at 37° C. for 2 hours, examining at 30 minute intervals for the formation of precipitate at the juncture of antiserum and antigen. This is the *precipitin ring test*.
- Shake the tubes to thoroughly mix the antiserum and antigen, and store overnight in the refrigerator.
- Next day the precipitate will have settled out and can be read by gentle agitation of the tubes.
- It is essential that controls of antigen plus saline, and antiserum plus saline, be included, and these should show no precipitate.

The precipitin test may also be carried out in capillary tubes to conserve antiserum, and to utilize the relatively small quantities of antigen available in bacterial extracts as in the typing of streptococci.

saline in certain proportions floccules appear, and the antigen is titrated to determine the smallest amount of saline which, when added to 1.0 ml. of antigen, produces aggregates which completely disperse upon the addition of more saline. Syphilitic serum has the property of preventing this dispersion. The test is based on the mixture of constant amounts

saline. A positive reaction is indicated by the presence of floccules in the mixture.

PROTOCOL FOR THE KAHN FLOCCULATION TEST

Tube Number	Antigen (ml)	Serum (ml.)	Serum Antigen Ratio	Shake vigorously for 2 minutes	Saline (ml)
1	0.05	0.15	3:1		0.5
2	0.025	0.15	6:1		0.5
3	0.0125	0.15	12:1		0.5

of a saline suspension, into the open mouth. The animals so treated were killed after intervals varying from a few hours to a month or more, and cultures were prepared from the blood, liver, spleen, mesenteric glands, the small intestine at different levels, and certain other situations. In this way it was possible to follow the spread of infection throughout the body. These observations are of particular interest because they are concerned with a natural disease of mice, and because the portal of entry is that by which the parasite gains access to its host in the natural spread of the disease.

It would seem that the bacilli, when they gain access to the body by the mouth, fail to establish any immediate foothold in the intestine, and for the most part rapidly succumb. A certain number, however, enter the tissues from the alimentary tract and are carried to the mesenteric lymph glands. Later they enter the blood stream, probably via the thoracic duct, but are rapidly removed from the circulation by the reticulo-endothelial cells, particularly those of the liver and the spleen. During this phase the blood taken from the heart is sterile, and *Salm. typhi-murium* cannot usually be recovered from the intestine; but the liver, spleen and mesenteric glands show its presence in increasing numbers. In many animals this stage is followed by a secondary bacteræmia, which increases in intensity until the animal succumbs. The intestine, which as stated above is rapidly freed from the invading bacteria during the primary stage of the infection, becomes secondarily infected during its later stages, probably by way of the bile-duct.

A similar series of experiments were carried out using *Salm. paratyphi B* as the infecting agent—an organism closely related to *Salm. typhi-murium*, but far less pathogenic for the mouse. The same rapid disappearance from the intestine was noted, and the same localization in the lymphatic glands; but in this case there appeared to be no tendency for the infection to spread beyond this primary focus, though the bacilli might persist in the mesenteric glands for weeks or months. A relatively avirulent variant of *Salm. typhi-murium* behaved in the same way as *Salm. paratyphi B*. These observations were extended by Maaloe (1948), who showed that a virulent strain of *Salm. typhi-murium* and an avirulent but nevertheless equally "endotoxic" variant were equally invasive, both reaching the mesenteric lymph nodes; but that the avirulent variant was destroyed there. Virulence was associated with *in vitro* resistance to the bactericidal action of fresh complement-containing serum.

The Antibacterial Action of Complement and other Humoral Substances.

Little is known about the *in vivo* importance of the bactericidal and bacteriolytic effects mediated by complement. Though purely humoral effects of this kind are probably less important than was at one time supposed, they are certainly operative with some organisms.

Thus, apart from Pfeiffer's classical experiments with *V. cholera*, Buxton and Torrey (1906d) note that many of the typhoid bacilli that collect on the surface of the omentum after an intraperitoneal inoculation are destroyed by extracellular lysis instead of being ingested by macrophages, and many workers have recorded analogous observations. We have just noted an example of an association of virulence and susceptibility to complement in *Salm. typhi-murium*, and there are other instances of this among Gram-negative bacteria. Moreover, in an experimental leptospiral infection of rodents, Stavitsky (1945) observed very little localization at the primary lodgment, and no evidence, either *in vitro* or *in vivo*, of phagocytosis of the organisms; he concluded that they were ultimately destroyed by lytic antibody. With Gram-positive organisms, like pneumococci or streptococci, it would seem, however, that this purely humoral mechanism plays no part. Besides complement, other humoral substances, either present in the tissue, or induced as the result of infection, have been reported as significant, or potentially significant, in antibacterial defences. These we consider in Chapter 52.

Agglutination. Bacteria, and other particulate antigens, aggregate in clumps in the presence of homologous antiserum, and are said to be agglutinated. The agglutination reaction is useful in the serological identification of bacteria, especially those of the group of enteric bacilli such as the *Salmonella* and dysentery bacilli. It is also the immunological reaction made use of, in conjunction with agglutinin absorption, in the antigenic analysis of bacteria.

The agglutination reaction differs from the precipitin reaction in that the antiserum rather than the antigen is diluted, and the antibody titer of the antiserum is expressed as the highest dilution in which a constant amount of bacterial antigen is agglutinated. Agglutinating antisera commonly show titers ranging from 1:1000 to 1:10,000, and occasionally may reach titers as high as 1:50,000 to 1:100,000 though this is rare. The agglutination of bacteria may be observed either microscopically or macroscopically, the former is generally used for typing purposes and the latter in the titration of antibody.

The Microscopic Agglutination Test. In the microscopic agglutination test either only a few, e.g., two or three, dilutions of antiserum are used, or, more commonly, a single dilution is used which preliminary titration has indicated will produce a rapid and com-

single rapid
right inocu
lating wire add a small amount of bacterial growth. The suspension should be faintly turbid and when this point is reached, the needle is flamed and then used

(2)

(3)

original dilution.

(4) Mount the cover slip on a hollow ground slide with petroleum jelly as in making a hanging drop preparation. No special incubation is necessary, and in a few minutes to an hour at room temperature agglutination occurs.

(5) The preparation may be examined under the low power objective for a curdled appearance, or under high power or oil immersion objectives for direct observation of the agglutinated bacteria. False clumping may occur and is distinguished from true agglutination in that in the latter all of the bacteria are gathered in large clumps with none about the edges of the drop, but false clumping occurs in small aggregates about foreign particles and around the edge of the drop

Macroscopic Agglutination. The titration of agglutinin is carried out in a manner similar to the titration of precipitin except, as noted above, with varying amounts of serum in the presence of a constant amount of antigen.

(1) PREPARATION OF ANTIGEN

- (a) Inoculate an agar slant culture with the bacteria to be used as antigen, spreading the inoculum over the entire surface of the slant.
- (b) After 18 hours' incubation run about 2 ml. of saline solution on the agar slant culture. The saline may contain 0.5 per cent formalin if a formalinized antigen is to be used.
- (c) Rub up the bacterial growth with an inoculating needle or loop to give a uniform suspension of the bacteria in the saline.
- (d) With flamed forceps remove a small piece of cotton from the bottom (sterile) part of the cotton plug of the culture tube and drop it into the saline suspension.
- (e) With a sterile pipette push the piece of cotton to the bottom of the saline, taking care to leave the cotton over the pipette opening.
- (f) Draw up the bacterial suspension into the pipette through the cotton, which serves to filter out coarse particles, and add this suspension slowly to the proper

slightly virulent, strain of the same bacterial species. Without any attempt to recapitulate in detail, we may quote a few illustrative examples.

Wright (1927) studied the response, to the intravenous injection of virulent pneumococci, of rabbits that had been immunized by the injection, at various intervals before the test inoculation, of a killed culture of the same strain. Table 69 and Fig. 245 show the results obtained in two rabbits that had been immunized 3 months previously, and in two normal controls injected with the same dose of the same living culture. Comparison with Table 68 and Fig. 244 will show that the immunized rabbits dealt with the highly virulent culture in the same way as the normal rabbit dealt with the slightly virulent strain.

TABLE 69

SHOWING THE NUMBER OF PNEUMOCOCCI PER ML. OF CIRCULATING BLOOD AT VARIOUS TIMES AFTER INOCULATION OF A VIRULENT STRAIN INTO NORMAL AND INTO ACTIVELY IMMUNIZED RABBITS.

Time after Injection	Normal		Immunized.	
	Rabbit 247.	Rabbit 248.	Rabbit 299.	Rabbit 300.
Immediately	870,000	1,100,000	1,000,000	1,000,000
5 hours	1,300	3,300	12	68
24 "	142,000	1,953,000	0	289
48 "	2,800	Innumerable	149	70
96 "	Dead	Dead	0	0

The phagocytic reactions that we have described are illustrated in Figs. 246, 247A, 247n and 248, for which we are indebted to the late Professor H. D. Wright.

The reaction in the lung capillaries in these immunized animals is illustrated by the following figures (Wright 1927) obtained from a film preparation.

Total number of pneumococci seen	893
Phagocytosed by polymorphonuclear cells	297
Phagocytosed by mononuclear cells	340
Total phagocytosed	637
Total outside phagocytes	256
In unphagocytosed aggregates associated with platelets	206
In free aggregates	53

Kerby, Holland and Martin (1950) measured the rate of removal of *Bact. friedlanderi* slowly infused into the inferior vena cava. During the first four hours it was, on their scale of measurement, of the order of 40 per cent., and was increased to 70 per cent. or more when the animals had been actively immunized by a similar perfusion 2 weeks previously, or passively immunized with plasma from another immune animal. Specific immunization greatly enhances the capacity of the rabbit to clear the blood of intravenously injected rickettsiae (Bieling 1949).

The results obtained when immunized animals are injected with virulent bacteria by other routes—by intraperitoneal or subcutaneous injection or by the mouth—are in entire conformity with those described above. An immunized animal reacts to a highly virulent bacterium as does a normal animal to one of lower virulence. The exact degree of difference in behaviour depends on the grade of immunity that has been established.

We may cite as further examples illustrating this important generalization about the reactions of normal and immune animals the studies of Angevine (1936) on intradermal infections by virulent streptococci; those of Jawetz and Meyer (1944a, b; see also Walker *et al.* 1953) on the reactions of the guinea-pig to infection by plague bacilli; and those of Downs and her colleagues on infections of mice and rats with *Brucella tularensis*

and if thought desirable of the general population, exclusion of contacts from schools, factories and other places of congregation, and careful observation of all doubtful cases of disease. Care should be taken over the treatment of the crusts, since they contain the variola virus, and may easily be disseminated in the form of dust.

Inoculated cutaneously into a fully susceptible human subject, vaccinia virus gives rise, after an incubation period of 3-4 days, to a papule which on the 6th or 7th day becomes vesicular. The vesicle, which is multilocular, umbilicated, and surrounded by a red areola, progresses to the pustular stage between the 10th and 12th days, after which retrogression sets in and the pustule dries up. The crust becomes detached about the 21st day, leaving a red pitted scar—the so-called foveation—which in the course of years turns white. The size of the reaction is dependent on the concentration of virus in the lymph (von Gröer 1935). A rise in temperature may occur about the 4th day, reaching its maximum about the 8th day; there may also be some enlargement and tenderness of the regional lymph nodes. A second inoculation made some years later may be followed by the usual *primary* type of reaction, or it may be accelerated in its evolution, though not in its appearance—*vaccinoid reaction*—the maximum being reached between the 3rd and 7th days. In persons recently vaccinated the so-called *immune reaction* is common; this consists of a papule or shallow vesicle reaching its maximum size in 8-72 hours and disappearing without passing through the pustular stage or leaving a scar.

There is some difference of opinion about the interpretation of the immune reaction. Broom (1947), who inoculated both fresh lymph and lymph inactivated by heating to 65° C. for 30 minutes into the same subjects, found that 110 out of 119 persons giving an immune reaction to the fresh lymph reacted also to the heated lymph. These results suggest that the reaction of immunity is essentially an allergic reaction (see also Mitman 1952). For this reason many workers prefer the term *immediate* to *immune* (Benenson 1950). Even this term is inaccurate, as the immune vaccinal reaction differs from the typical immediate reaction of the wheal-and-erythema type met with, for example, in hay fever. The best term, we think, by which to describe it is the *allergic* reaction. Though this reaction may persist long after immunity has disappeared, it is nevertheless true to say that, provided a lymph of proved activity is used, an allergic reaction does probably mean that the subject possesses a considerable, though by no means necessarily complete, immunity to vaccinia, and inferentially to smallpox. Otherwise it would be difficult to understand why in Broom's series the proportion of primary reactions rose and of immune reactions fell in relation to the length of time elapsing since previous vaccination.

For human vaccination calf or sheep lymph is generally employed, but lymph taken from vaccinal lesions in other animals may be used satisfactorily. Bacterial contamination can be largely controlled by phenol and glycerol (McClellan 1919) or by one of the quaternary ammonium compounds (Ducor 1947). If stored in the dark below 0° C vaccine lymph should retain its potency for at least 6 months; if between 0 and 10° C. for 14 days; if at room temperature (20° C.) for not more than 7 days. Of late years two other types of vaccine have been introduced, partly to provide a bacteria-free product, partly to avoid the severe local and constitutional reactions to which calf lymph sometimes gives rise, and partly in the hope of preparing a vaccine of constant virulence. The first is the so-called Rivers vaccine,

glutination, from + or barely perceptible partial agglutination to ++++ of complete agglutination with a clear supernatant, while others simply record the presence or absence of agglutination with a single + sign. The agglutinin titer of the antiserum is the highest dilution in which definite agglutination occurs.

Complement Fixation. The complement-fixation test is based on the observation that complement, a heat-labile constituent of normal serum, combines with an antigen-antibody complex and is said to be fixed. Since this fixation produces no visible change, a hemolytic system, consisting of sheep erythrocytes and anti-sheep cell hemolysin, is added. If the complement is free, *i.e.*, has not been fixed, and inferentially the original union of antigen and antibody has not occurred, the erythrocyte-hemolysin complex combines with the complement, and visible lysis of the red cells occurs. Conversely, if the erythrocytes are not lysed, the test antigen-antibody system has combined and fixed the complement, leaving none for hemolysis. This complement-fixation test is most often applied in the serodiagnosis of syphilis, but has proved very useful with the rickettsiae. Bacterial antigens fix complement readily.

The following reagents are required:

- (1) The antigen antibody system to be tested, either component of which may be unknown.
- (2) Anti-sheep erythrocyte hemolysin, prepared by the immunization of rabbits with sheep red cells.
- (3) A 2 per cent (by volume of packed cells following centrifugation) suspension of washed sheep erythrocytes in saline.
- (4) A source of complement, practically always fresh guinea pig serum.

Titration of Reagents Since the test is necessarily quantitative with respect to the relative proportions of the various components, the activity of these must be titrated prior to the actual test. These preliminary titrations include.

- (1) The titration of hemolysin which is carried out by varying the amount of hemolysin in the presence of constant amounts of sheep erythrocytes and complement, the last in amounts more than necessary for complete lysis. This titration is carried out as indicated in the accompanying protocol. The smallest amount, *i.e.*, highest dilution, of hemolysin required to bring about complete lysis is the *hemolytic unit*. In the final test 2 units in a volume of 0.5 ml. are used in each tube and on the basis of this titration the hemolysin is diluted to contain 4 units/ml.

PROTOCOL OF HEMOLYSIN TITRATION

Tube Number	Hemolysin Dilution (0.5 ml.)	Complement 1:10 Dilution (ml.)	2% Suspension Erythrocytes (ml.)	Saline (ml.)
1	1:1000	0.3	0.5	1.7
2	1:2000	0.3	0.5	1.7
3	1:3000	0.3	0.5	1.7
4	1:4000	0.3	0.5	1.7
5	1:5000	0.3	0.5	1.7
6	1:6000	0.3	0.5	1.7
7	1:8000	0.3	0.5	1.7
8	1:10,000	0.3	0.5	1.7
control (9)*	1:1000	none	0.5	2.0
control (10)*	none	0.3	0.5	2.2

* both controls must be negative

(3) The greater the area of cicatrix left by the vaccination, the greater is the diminution of the fatality rate (see Table 173).

According to Turner (1905-06), among cases with equal total areas of vaccination scarring there is no difference in the amount of protection afforded by different numbers of scars. Hanna (1913) at Liverpool, who made observations on

TABLE 171

Year.	Vaccinated.			Unvaccinated or Doubtful.		
	Admitted.	Died.	Case-fatality rate per cent.	Admitted.	Died.	Case-fatality rate per cent.
1901 . . .	1,282	127	9.9	461	165	35.8
1902 . . .	5,663	578	10.2	2,253	759	33.6
1903 . . .	245	7	2.9	110	5	4.5
1904 . . .	289	14	4.8	160	13	8.1
Totals . .	7,479	726	9.7	2,984	942	31.57

TABLE 172

	Admitted	Died.	Case fatality rate per cent.
With 4 scars or more . . .	3,139	171	5.4
" 3 " . . .	1,902	171	9.0
" 2 " . . .	1,442	184	12.8
" 1 scar . . .	930	168	17.9

TABLE 173

Area of Scar.	Admitted.	Died.	Case-fatality rate per cent.
Area of $\frac{1}{2}$ sq. in. or more . .	5,564	388	7.0
" $> \frac{1}{2}$ sq. in. but $< \frac{1}{4}$ sq. in.	889	138	15.5
" $< \frac{1}{2}$ sq. in. . . .	933	158	16.9

943 vaccinated persons suffering from smallpox, compared the area of scar tissue left by primary vaccination with the severity of the disease. From the information he gives we have worked out the following average figures:

Modified discrete, and discrete	0.728 sq. in.
Profuse discrete, and semi-confluent	0.670 sq. in.
Confluent and death	0.456 sq. in.

These figures support those collected by Cameron and others in showing the inverse relationship between the area of scar tissue and the severity of the disease.

(4) In vaccinated persons the fatality from smallpox increases with the length of time elapsing since vaccination. Table 174 affords a comparison, in this respect, between vaccinated and unvaccinated persons. This table also shows a very different age-grouping of admissions in vaccinated and unvaccinated persons,

PROTOCOL OF COMPLEMENT TITRATION

Tube Number	1:30 Dilution Complement (ml.)	Saline (ml.)	Hemolysin + Units/ml (ml.)	2% Suspension Erythrocytes (ml.)
1	0.10	1.9	0.5	0.5
2	0.15	1.9	0.5	0.5
3	0.20	1.8	0.5	0.5
4	0.25	1.8	0.5	0.5
5	0.30	1.7	0.5	0.5
6	0.35	1.7	0.5	0.5
7	0.40	1.6	0.5	0.5
8	0.45	1.6	0.5	0.5
9	0.50	1.5	0.5	0.5
control (10)*	0.00	2.5	0.0	0.5

* control must be negative

- (2) The complement of fresh guinea pig serum is similarly titrated, using variable amounts of complement in combination with constant amounts of erythrocytes and

ment fixation test 2 full units in a volume of 1.0 ml are used in each tube. The reason for the excess amount is that some complementary activity is lost during the time allowed for fixation, even if no fixation occurs, and the test is sufficiently sensitive even with this slight excess.

- (3) Certain properties of the antigen are pertinent to the test and must also be determined by preliminary titration. These properties include anticomplementary activity, i.e., inhibition of the action of complement, hemolytic activity, and binding power for complement. The titration of these is shown in the accompanying protocol. In general, the binding power of the antigen should be at least 10 times its anticomplementary action, and in the final test not more than one third of the amount of antigen found to be anticomplementary may be used. It is self-evident that the antigen must not lyse red cells in the concentrations used in the test.

Procedure of the Test:

- (1) Inactivate the test serum, i.e., destroy any traces of complement in it, by heating to 56° C. for 15 to 20 minutes, and dilute 1:5, and distribute in three 15 x 100 mm. test tubes as indicated in the accompanying protocol. Bring the total volume in tubes 2 and 3 to 0.5 ml. by adding 0.25 and 0.375 ml. of saline respectively.
- (2) Add 0.5 ml. of antigen, diluted to contain 20 complement fixing units per ml. to each tube.
- (3) Let the mixture stand at room temperature for 10 minutes.
- (4) Add 1.0 ml. of complement, diluted to contain 2 full units per ml., to each tube.
- (5) The mixture may be incubated in a water bath at 37° C. for 1 hour, or stored in the refrigerator overnight and warmed in the water bath for 10 to 15 minutes before proceeding with the test the following day. This time interval is allowed for fixation of the complement by the antigen antibody complex, and the latter method is referred to as "ice box fixation."
- (6) Add 0.5 ml. of hemolysin diluted to contain 4 hemolytic units per ml. to each tube.
- (7) Add 0.5 ml. of a 2 per cent suspension of washed sheep erythrocytes to each tube.
- (8) The appropriate controls of the serum, antigen, hemolytic system and erythrocytic suspension are indicated in the protocol, and must be included.
- (9) Incubate in a water bath at 37° C. for 15 to 60 minutes.
- (10) Read the hemolysis as complete (+++), partial (+++, ++, +, ±) or negative.

after birth to enable the passive immunity received from the mother to wear off (Béclère 1936). (For general discussion of vaccination, see Greenwood 1930, 1935, Report 1931, Russell 1941, Robinson 1941, Stevenson 1944, Downie 1951b)

Complications of Vaccination.—Of the complications that may follow vaccination, suppuration is one of the commonest, and tetanus one of the most serious. Owing to the use of calf lymph, which cannot be satisfactorily sterilized, the introduction of micro-organisms into the wound is inevitable, but it is very difficult to say how often the calf lymph is responsible, and how often uncleanness on the part of the operator, or during the after-treatment. Occasionally, as at Malmo, severe necrotic lesions may occur owing to the presence of pathogenic staphylococci in the lymph (Magnusson 1932, 1933). It may be noted that foot-and-mouth virus has been found occasionally in specimens of calf lymph, though there is no evidence that its presence excited any unfavourable reaction in vaccinated children (see Gildemeister 1931, Gildemeister and Helm 1932*a*, *b*).

Tetanus is a very rare complication; thus in 1904–13 in the United States, 31 million lymph doses were inoculated into human beings, and only 41 cases of tetanus were observed. Infection in these cases may be carried by the lymph; but tetanus occurs only in those cases in which a protective shield or dressing strapped to the arm has been applied. Vaccination insertions treated openly have never been followed by tetanus (Armstrong 1927).

Generalized vaccinia is a serious complication, which is estimated to occur in 1 out of every 30,000 subjects vaccinated. Persons with chronic skin lesions are said to be specially susceptible. The disease varies from a mild rash with a few scattered vesicles and little constitutional disturbance to a severe variola-like disease with tracheitis, general glandular enlargement, widespread affection of the skin, and a rapid course to death. The case fatality is said to be 30–40 per cent. (see Ross 1941)

Another complication, which is very serious, is *post-vaccinal encephalomyelitis*. The first case of this to be studied in detail was by Turnbull in 1912, but it was not till the publication of Turnbull and McIntosh's report in 1926 that serious attention was drawn to the subject. Clinically, after an incubation period of 10–12 days, with a range of 2 to 28, there is a rapid onset followed by an acute course, the symptoms being cerebral rather than spinal (Report 1928*a*). Histologically, the lesions are widely distributed in the grey and white matter of the brain and cord and are characterized particularly by extra-vascular parenchymatous infiltration with large endothelial and glial cells and by perivascular areas of softening or demyelination (McIntosh 1928). The incidence varies in different countries and at different ages (Stuart 1947–8, Conybeare 1948, Greenberg and Appelbaum 1948), but exact figures are difficult to obtain. The figures quoted by different observers range from about 1 in 1,000 to 1 in 100,000 vaccinated persons. The incidence rate is very low in infants under 1 year of age; it reaches its maximum during the school period, and declines again in adult life. On the other hand, the case-fatality rate is usually highest in infants, intermediate in school children, and lowest in adults. The average for all ages is about 50 per cent. Most cases have occurred after late primary vaccination, but sometimes the disease has followed revaccination—usually performed several years after primary vaccination. There seems to be no constant relationship between the severity of the vaccinal reaction and the liability to develop the disease. The best method of preventing this complication is to perform primary vaccination when the infant is about 3 months old,

PROTOCOL OF ANTIGEN TITRATION

Property Tested	Tube Number	Antigen* (ml.)	Antiserum* 1:25 Dilution (ml.)	Complement 1:10 Dilution (ml.)	Saline (ml.)	37° C. for 1 hour or store in refrigerator overnight		Hemolysin 4 Units/ml. (ml.)	2% Suspension Erythrocytes (ml.)
Anticomplementary Activity	1	0.5	0	0.3	0.2			0.5	0.5
	2	0.4	0	0.3	0.3			0.5	0.5
	3	0.3	0	0.3	0.4			0.5	0.5
	4	0.2	0	0.3	0.5			0.5	0.5
	5	0.1	0	0.3	0.6			0.5	0.5
	6	0.05	0	0.3	0.65			0.5	0.5
Hemolytic Activity	7	0.5	0	0	1.0			0	0.5
	8	0.1	0	0	1.4			0	0.5
Complement-binding power	9	0.5	0.25	0.3	0.05			0.5	0.5
	10	0.25	0.25	0.3	0.2			0.5	0.5
	11	0.1	0.25	0.3	0.35			0.5	0.5
	12	0.075	0.25	0.3	0.375			0.5	0.5
	13	0.05	0.25	0.3	0.4			0.5	0.5
	14	0.025	0.25	0.3	0.425			0.5	0.5
Controls	15†	0	0.25	0.3	0.45			0.5	0.5
	16‡	0	0.25	0	1.75			0	0.5

* The amounts of antigen and antiserum given here are arbitrary and will vary with individual preparations

† This control should show complete hemolysis

‡ This control should show no hemolysis

analysis. There is however no doubt that these viruses are all very alike, and are able to afford almost complete cross-protection between each other (see Borrel 1903, Bosc 1903, de Jong 1917, Zwick 1924, Toyoda 1924, von Wasielewski and Winkler 1925, Ledingham 1926, Findlay 1936).

One interesting property of the pox viruses is their power to give rise in certain hosts—sheep and fowls particularly—to tumours characterized by epitheliomatous proliferation superficially resembling neoplasms.

Cow-pox is a mild disease affecting the teats and udders. In its clinical form it is not very common, though there is reason to believe that the virus often gives rise to subclinical and latent infections. The disease is transmissible to man, and in unvaccinated persons generally runs a more severe course than that characteristic of vaccinia. The contents of the vesicles both in man and animals may be blood-stained. Histologically, strongly acidophilic compact intracytoplasmic inclusion bodies are seen in the epithelial cells differing from those of vaccinia and variola. Minor serological differences can be demonstrated between cow-pox and vaccinia virus, but each protects against the other and against variola (see Downie 1951b).

Sheep-pox, known in France as *la clavelée*, is of some economic importance. It was studied by Borrel in 1903 who noted the occurrence of distinctive cellular bodies in the lesions, and showed that the causative agent was able to pass through coarse Chamberland filters. The virus was cultured by Gins and Kunert (1937) on the chorio-allantoic membrane of the chick embryo.

The disease, which runs an acute febrile course, is characterized by the development of a vesicular eruption, later becoming pustular, on the more exposed parts of the body. Infection can readily be transmitted to normal sheep by inoculation with the serous fluid contained in the loculated vesicles. The case fatality of the natural disease is very low. Post-mortem examination of fatal cases reveals the presence of generalized lesions. The lungs contain multiple, small, dense, spherical nodules, hyaline in appearance with a central opaque spot; they are commonest near the pleural surface. Similar, but less obvious, lesions are also seen in the liver and kidneys. Prophylactic inoculation with a sensitized vaccine is widely practised in France (Bridré and Boquet 1923, 1933). Inoculation with vaccinia virus is said to protect sheep against sheep-pox (Gins and Kunert 1937).

Rabbit-pox was studied by Pearce and her colleagues (Pearce *et al.* 1936a, b, Rosahn *et al.* 1936a, b, Hu *et al.* 1936). The virus is filtrable through Berkefeld V candles; it is antigenically related to the vaccinia virus, but not to Virus III (see p. 2141), or the virus of infectious myxoma (see p. 2243); and it is able to infect guinea-pigs, mice, calves, and probably rats.

Infectious Ectromelia or Mouse-pox is a disease of mice first described by Marchal (1930) at Hampstead. It occurs in an acute form characterized by the presence of visceral lesions, in a chronic form characterized by necrosis of the feet, and in a latent symptomless form. In the chronic form one of the feet becomes swollen and oedematous, and serous fluid escapes from the surface, forming minute scabs. The disease may retrogress, in which case the foot becomes gangrenous and sloughs off leaving healthy tissue behind; or infection may spread to another foot, the tail, or the mouth, and lead to the death of the animal. In the acute form the animals die without any external lesions. *Post mortem* the liver may be dirty grey in colour, or it may be red and mottled due to the presence of small white focal areas of necrosis. The spleen is slightly enlarged, and may show large white areas.

PROTOCOL OF THE COMPLEMENT FIXATION TEST*

Tube Number	Serum Diluted 1:5 (ml.)	Antigen 20 Units/ml. (ml.)	Saline (ml.)	Let stand at room temperature for 10 minutes.	Complement 2 Units/ml. (ml.)	Hemolysin 4 Units/ml (ml.)	2% Suspension Erythrocytes (ml.)
1	0.5	0.5	0		1.0	0.5	0.5
2	0.25	0.5	0.25		1.0	0.5	0.5
3	0.125	0.5	0.375		1.0	0.5	0.5
4 (SC)	0.5	0	0.5		1.0	0.5	0.5
5 (AC)	0	0.5	0.5		1.0	0.5	0.5
6 (HC)	0	0	1.0		1.0	0.5	0.5
7 (EC)	0	0	2.5		0	0	0.5

* Set up as the Kolmer modification of the Wassermann test

SC serum control—should show complete hemolysis

AC antigen control—should give complete hemolysis

HC hemolytic system control—should give complete hemolysis

EC erythrocyte control—should give no hemolysis

sequence of events may probably be accepted as a prototype for the behaviour of the variola virus (see p. 2112).

Fowl-pox requires separate consideration. According to Doyle (1929) three clinical forms may be recognized: (1) wart-like nodules on the comb, wattles, and skin of the head; (2) adherent, yellow, cheesy membranes in the mouth; (3) a watery or muco-purulent discharge from the eyes and nose; this form is sometimes referred to as "roup." One, or any combination, of these forms, may be present in the same bird.

The fowl-pox virus is said to be brick-shaped and slightly larger than vaccinia virus, measuring about $332 \times 264 \text{ m}\mu$ (Boswell 1947). Though Toyoda (1924) maintained that by passage through rabbits the fowl-pox virus could be converted into the vaccinia virus this seems very doubtful (Findlay 1928). Both in its pathogenicity and in its serological reactions it seems to be less nearly related to vaccinia than are the mammalian pox viruses. In the lesions of fowl-pox so-called Bollinger inclusion bodies, and Borrel elementary bodies, corresponding to the Guarnieri corpuscles and the Paschen bodies of vaccinia, can be found (see Ludford and Findlay 1926).

For protection against the disease pullets may be vaccinated during the first 4 or 5 months of life with virulent fowl-pox virus (see Beaudette 1949). Alternatively the pigeon-pox virus may be used. Doyle and Minett (1927) found that pigeons were resistant to fowl-pox but that fowls were susceptible to pigeon-pox. They obtained evidence moreover that pigeon-pox protected fowls against fowl-pox. The vaccine is prepared with dried pigeon-pox crusts suspended in glycerolated saline. The advantage of this vaccine is that it can be used at any time of life.

Kikuth and Gollub (1932) described a disease of canaries, which appears to be **canary-pox**. It was studied by Burnet (1933) and Burnet and Lush (1936a), who found that the clinico-pathological picture differs from that of fowl-pox, but that the viruses of the two diseases are antigenically closely related.

CONTAGIOUS PUSTULAR DERMATITIS OF SHEEP

Contagious pustular dermatitis or Orf (from a Saxon word for cattle) is a highly infectious disease of sheep. It occurs not infrequently in this country and Australia, and has been described by several workers on the Continent (for references see Glover 1928). It is probable that natural cases occur also amongst goats. The disease varies in severity: in the mild form there are pustular lesions on the lips and surrounding tissues; in the severe form lesions occur on the oral mucosa, the vulva, the cornea, and on various other parts of the body, particularly the regions of the coronet and the tail. Sheep of any age may be attacked, but the disease is commonest in weaned lambs up to 1 year old. Experimentally vesico-pustular lesions can be produced by scarification of the thighs of normal lambs, or by intradermal inoculation of the lip with a suspension of dried crusts from the natural lesions; the crusts generally prove active in a dilution of 1/50,000. Elementary bodies may be found in the vesicular fluid (Lloyd *et al.* 1951). The disease is not readily transmissible to laboratory animals, but some strains can be adapted to the rabbit, in which they produce a papillomatous form of lesion (Selbie 1944). The causative virus is of much the same size as that of vaccinia, by the centrifugal method it appears to be at least $150 \text{ m}\mu$ in diameter (Selbie 1945). It withstands

MORPHOLOGY, CELL STRUCTURE, GROWTH AND CHEMICAL COMPOSITION OF BACTERIA

Bacteria are widely distributed over the surface of the earth—in the soil, in the sea and in fresh water, and on the bodies of plants and animals. The true bacteria constitute only a portion, though a very large portion, of this vast microbial population, the remainder consisting of unicellular protozoa and fungi, including yeasts, molds and actinomycetes. This microbial population far exceeds, in point of numbers of individuals, that of all macroscopic plants and animals combined. So ubiquitous are the bacteria that there are few places to which man has access where they may not be found. They are present in the upper atmosphere and deep in the earth and sea. With the exception of certain obvious places such as the interior of active volcanoes, they may be said to occur universally over the surface of the earth. The skin of animals is covered with, and their gastro-intestinal tracts contain, tremendous numbers of these organisms. It is often stated that bacteria are absent from the tissues of healthy animals, but it appears that this is not always true.

As the discovery of this microbial world necessarily awaited the development of optical systems which made bacteria visible, so the structure of our knowledge of these organisms is built upon concepts derived from direct observation. Although the morphology of bacteria will not carry us far in their taxonomy, nor will it give us any insight into their physiological activities, nevertheless knowledge of the size and shape of these microorganisms, their internal and external structures, their processes of division and other characteristics determinable by direct observation, is the solid foundation upon which bacteriology rests.

Size. Different kinds of bacteria vary considerably in size. They are ordinarily measured in terms of microns (μ). The micron, a unit of the metric system, is 0.001 millimeter or roughly 1/25,000 of an inch. The average bacterium of rod shape measures about 2 μ in length and 0.5 μ in diameter. One large spherical bacterium has been described that measures about 2 μ in diameter; the most common microbe found in suppurative processes is a spherical bacterium about 0.8 μ in diameter. The largest bacteria belong, as a rule, to the group of spirally twisted or screw-shaped forms¹; one of these² has been found to measure as much as 35 μ in diameter. Perhaps the largest pathogenic bacterium is the spirochete of relapsing fever, which may measure

¹ A bacillus (*B. butschlii*), however, studied by Schaudinn (Arch. f. Protistenk., 1902, 1:306) measures from 50 to 60 μ in length and from 4 to 5 μ in width.

² *Spirillum colossus* (Centralbl. f. Bakt., 1902, Abt. II, 9 608).

(Downs *et al.* 1949, Buehler and Downs 1949, Downs and Woodward 1949). It applies not only to clearance from the blood, but also to disappearance of the bacteria from the viscera and the lymphatic system.

The immunity with which we are here concerned is, it should be noted, strictly specific. The influence of non-specific factors on immunity is considered in Chapter 52.

The Passive Transference of Antibacterial Immunity.—The increased resistance that an actively immunized animal enjoys in virtue of the increased efficiency of the defence mechanism considered above can be passively transferred from an immunized to a normal animal by injecting into the second the blood serum of the first.

It follows that we must regard the mechanism by which virulent bacteria are disposed of, when once they have gained access to the tissues of an immunized animal, as an integrated system of cellular reactions conditioned by the presence of specific antibodies in the blood or tissue fluids.

Again, a few examples will suffice.

Bull (1915*a*, *b*) found that, in a rabbit in which a bacteraemia had been established by the intravenous injection of pneumococci, the bacteria were removed from the circulation within about 15 minutes after the intravenous injection of 0.2-0.5 ml. of antipneumococcus serum per kilo-body-weight of the rabbit.

Wright (1927) showed that the clearing mechanism of a normal rabbit could be rendered highly effective against virulent pneumococci by the intravenous injection of the serum of an actively immunized animal. The results of one such experiment are set out in Table 70 and Fig. 249. The two passively immunized rabbits each received 1 ml. of the serum of an actively immunized rabbit 1 hour before the test dose of culture. It will be noted that the normal animal died between the 5th and 24th hours, so that the expected secondary rise in the number of bacteria was not observed.

TABLE 70

SHOWING THE NUMBERS OF PNEUMOCOCCI PER ML. OF CIRCULATING BLOOD AT VARIOUS TIMES AFTER INOCULATION OF A VIRULENT STRAIN INTO ONE NORMAL AND TWO PASSIVELY IMMUNIZED RABBITS.

Time after Injection.	Normal	Pass. Imm. 1.	Pass. Imm. 2.
Immediately	2,300,000	2,300,000	2,000,000
5 hours	43,000	2	52
24 "	Dead	8	14
48 "	—	0	1
96 "	—	0	0

Manwaring and Coe (1914) perfused a normal liver with a dilute suspension of pneumococci, with and without the addition of an antipneumococcal serum, and found that the retention of pneumococci within the liver capillaries was pronounced in the former cases but very slight in the latter.

There are many earlier observations of an analogous kind.

Bordet (1897) showed that an antistreptococcal serum would protect a guinea-pig against the intraperitoneal injection of a lethal dose of living streptococci. By withdrawing samples of the peritoneal exudate at various intervals after the inoculation, he was able to show that the recovery of the passively immunized animal was associated with a greatly increased degree of phagocytosis, mainly by the polymorphonuclear cells. By a very ingenious experiment he demonstrated that the relatively slight degree of phagocytosis in the peritoneal exudate of the normal animal could not be ascribed to an absence of activity on the part of the leucocytes. If a suspension of *Proteus* bacilli was injected

up to $40\ \mu$ in length. One of the smallest of the well-known pathogenic forms is the so-called "influenza bacillus," which is about $0.5\ \mu$ by $0.2\ \mu$.

Not only does the size of bacteria vary considerably from species to species but there is also great variability within a single species—much greater than one finds among many other forms of life. The bacillus of typhoid fever is found to range from 1 to $3\ \mu$ in length even when the descendants of a single cell, living under substantially identical conditions, are examined.

There is reason to suppose that still smaller organisms may exist. The infective agents of smallpox, infantile paralysis and a number of other diseases cannot be seen with the ordinary microscope and are so small that they will pass through filters that remove all known visible bacteria. These infective agents—known as the viruses or filterable viruses—are believed by many to be minute living organisms incapable of multiplication except in the presence of living host cells. It is open to question as to whether the viruses are related to ordinary bacteria. Measurements made by means of filtration through graded collodion membranes, which allow particles of known size to pass, indicate that the particles of certain viruses are as small as $0.01\ \mu$ in diameter.³

The mitochondria of living cells have been supposed by some investigators to be symbiotic bacteria, visible forms which are parasitic on the host cell as the viruses apparently are. However, microchemical and physical differences between bacteria and such structures render such a supposition unlikely.⁴

Morphology. For practical purposes the morphology of bacteria is best considered under two heads: the morphology of individual cells and groups of cells, or microscopic morphology, and the morphology of large aggregates of cells in bacterial colonies, macroscopic or colonial morphology. The individual cells differ in size, shape and other structural details, features which can be determined only by the use of high magnification. Colonies or masses of cells that develop on the surface of solid media often present peculiarities of form, color, consistency and the like which are apparent upon examination with the naked eye or low power lenses. As will appear, there is a relation between colonial and cellular morphology. Analogous differences may be observed between masses of larger objects which are associated with the appearance of the single objects. A grove of oak trees viewed from a distance too great to permit identification of the individual trees will still appear unlike a grove of pine trees.

Shape. With respect to shape, bacteria exist in three principal types: the spherical or coccus form; the rod-shaped form known as the bacterium or bacillus, and the spiral forms, the various subtypes of which are designated as vibrio, spirillum and spirochete.

Coccus. The cocci may be subdivided on the basis of the positions which the individual cells tend to take with respect to one another. Such groupings are primarily a result of the planes in which cell division takes place and the behavior of the daughter cells after division is complete. Those organisms which separate completely after cell division and appear singly and scattered at random over the microscopic field are designated as *micrococci*. In some

³ The millimicron ($m\mu$) is often used as the unit for such measurements. One μ is equal to 1000 $m\mu$.

⁴ Cowdry and Olitsky. *Jour. Exp. Med.*, 1922, 36: 521.

Buddingh and Dodd (1944) described a form of stomatitis in infants characterized by numerous fine vesicles on the mucosa of the tongue and gums sometimes associated with diarrhoea and caused by a virus differing from that of herpes.

Inoculation of the fluid from a herpes vesicle on to the scarified cornea of a rabbit leads to the development of a severe keratitis, which can be propagated indefinitely by passage. If fluid is taken from an early keratitis in the rabbit, it is possible to produce with it specific lesions in a number of different tissues, such as the skin, buccal mucosa, trachea, liver, adrenal, ovary, salivary glands, brain, and spinal cord (Goodpasture and Teague 1923-24a, Urbain and Schaefer 1927, Smith 1931a). Intracutaneous inoculation of human vesicular fluid produces in the rabbit an insignificant papular eruption, but once it has become adapted to the rabbit's tissues, the virus gives rise to a local herpetic lesion; this method is of considerable value in the titration of infective material. Direct inoculation of human vesicular fluid on to the pad of the guinea-pig's foot is uniformly followed by the development of local vesicular lesions (Bedson 1934). A certain proportion of rabbits inoculated on the cornea develop, after a few days, a fatal encephalitis, characterized by widespread small-celled infiltrations, intense nerve-cell degeneration, and diffuse proliferative changes of some of the fixed elements of the tissue affected (da Fano 1923). Direct intracerebral or subdural inoculation of vesicular fluid into rabbits gives rise to a meningo-encephalitis which proves fatal in about 5 days, and which is transmissible in series to other rabbits by inoculation of the brain suspension.

Rabbit brain is highly infective, but its infectivity can be increased by homogenization—a process which probably acts by disintegration of the cells and liberation of the virus particles in a free form. Freshly homogenized virus may prove fatal on intracerebral inoculation into rabbits in 12-30 hours, and even one ten-millionth of a gram of infected brain tissue may kill the animals within 5 days (Buggs and Green 1936).

Experiments with monkeys have not given uniform results, but according to Teissier, Gastinel and Reilly (1925) cutaneous inoculation in *Callithrix* may result in the development of vesico-pustules, though often merely small papules appear. Corneal or intracerebral inoculations were without effect.

The virus can be passed from rabbits to mice by intracerebral inoculation with the brain of a rabbit suffering from herpes encephalitis, or with the ground-up nictitating membrane of a rabbit in which the corneal reaction is well established (Steigman and Scott 1942). Encephalitis develops in 3-10 days. Infection can be maintained indefinitely by passage through mice (Andervont 1929). With a neurotropic strain, cutaneous inoculation or intranasal infection of mice gives rise to encephalitis (Gildemeister and Ahlfeld 1936, Berry and Slavin 1943, Slavin and Berry 1943).

The herpes virus can be grown easily in tissue cultures, one of the most suitable media being rabbit testis (Parker and Nye 1925b, Gildemeister *et al.* 1929, Smith 1931b). It can also be adapted to grow on the chorio-allantoic membrane of the developing chick embryo. Since pocks are formed on the membrane, this method lends itself to the titration of virus and of neutralizing antiserum (Burnet 1936, Burnet and Lush 1939a, Shaffer and Enders 1939). In the vesicular fluid the virus is present in the form of elementary bodies, which can be stained by suitable means (Taniguchi *et al.* 1934). Examination of the corneal lesions in rabbits during the first 24 hours reveals the presence of large numbers of acidophilic intranuclear inclusion bodies—referred to as "α-bodies" by Lipschütz (1921) (Fig. 302).

species, however, there is a tendency for the individual cells to remain in pairs, and one may observe such pairs intermingled with individual cells. These organisms are called *diplococci*. When this pairing tendency is marked as, for example, in the gonococcus, the individual cells may not be perfectly spherical but exhibit a coffee bean shape with the concave sides facing each other. Often coccus forms tend to remain together in sheets or irregular clusters resembling bunches of grapes. Those which assume this grouping are termed *staphylococci*. Division in two or three planes is, of course, necessary to the formation of such groups. Other coccus forms divide in only one plane but tend to remain together, the result being a chain of cocci. Organisms forming such chains are

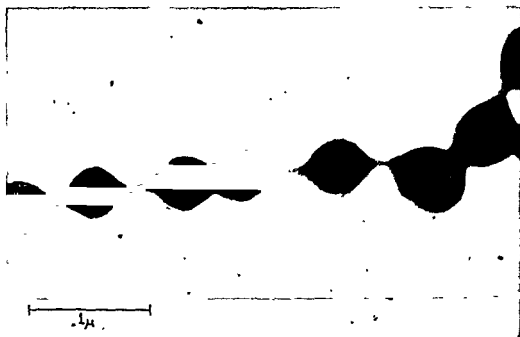


Fig. 2. Electron micrograph of *Streptococcus pyogenes*. Note the manner in which the individual cells remain attached to one another following fission, and the tendency of the chains to be made up of attached pairs of cells. (SAB No. 71.)

called *streptococci*. Still other cocci divide in three planes and remain together to form cubical packets of eight cells. These organisms are the *sarcinae*. Not all the cells in a given microscopic field will appear in characteristic groups. Many organisms may be found singly, presumably those which have broken away from the parent group. Chain formation by streptococci is influenced by the medium upon which the organisms have been grown. The acid-producing streptococci of milk, for example, show long chains in milk culture but when grown in nutrient broth may show only a few short chains. In smears of pus or similar material, however, the coccus forms are usually found in their characteristic groupings. Of the coccus forms having definite grouping, the staphylococci are the most consistently characteristic under the microscope.

Bacillus. The morphology of individual rod-shaped bacteria differs considerably from species to species. Not only is there variation in size but the shape of the individual cells differs. Some bacilli have sides more or less parallel with one another but rounded ends. Others have quite square ends which

and Burnet and Williams (1939), working in Melbourne, found neutralizing antibodies present in over 90 per cent. of children and adults of the hospital classes, but in only about 50 per cent. of members of the higher social classes. This they explain by the greater frequency of primary herpetic stomatitis in early life among children of the lower classes. Since recurrent herpes affects persons whose blood serum contains neutralizing antibodies, it is assumed that the virus persists in the body tissues—probably in the mouth (Scott and Steigman 1941)—and that immunity is to a considerable extent non-specific. The failure of the antibody to afford protection is curious. Herpes febrilis constitutes, in fact, one of the most striking exceptions to the general rule that filtrable virus infections usually leave behind them a solid and lasting immunity. A skin reaction that is said to be positive in persons subject to recurrent attacks of herpes was described by Nagler (1944).

Keratitis herpetica of human beings was shown by Grüter in 1912-14 to be transmissible to rabbits by inoculation of the scarified cornea. This observation was confirmed by Luger and Lauda (1921), who found, histologically, alterations in cellular structure similar to those in herpes febrilis. These consisted of giant-cell formation, the characteristic ballooning degeneration of Unna, and bodies which they considered to be due to a specific degenerative reaction of the nucleus, but which Lipschütz regarded as inclusion bodies.

Herpes genitalis has likewise been shown to be transmissible to rabbits, but the keratitis set up by the genitalis virus is much less severe than that set up by the febrilis virus. Similarly, on subdural inoculation the genitalis virus is less virulent; by this route the febrilis virus uniformly produces encephalitis in rabbits, fatal in 4 to 6 days; the genitalis virus does not uniformly produce encephalitis, and, when it does, the rabbits do not die for 5 to 17 days (Blanc and Caminopetros 1924). Lipschütz (1921) succeeded in transferring genital herpes to human beings. Both in the human lesions and in the rabbit's cornea, he found nuclear inclusion bodies of the so-called β -type present in large numbers; they are best looked for in the rabbit's cornea on the 3rd day after inoculation. He was unable to produce immunity to the genitalis virus by inoculation of the rabbit's cornea with the febrilis virus. He therefore regarded the two viruses as distinct.

Herpes zoster.

Herpes zoster differs clinically from herpes febrilis in forming large vesicles or bullæ, in occurring on the trunk, in being almost always unilateral, in being accompanied by sharp neuralgic pains, in being distributed along the course of sensory nerves, and in being non-recurrent. Experiments made to ascertain whether zoster is transmissible to animals have given most conflicting results.

Lipschütz (1921) claimed to have produced keratitis in rabbits with material from 3 out of 7 cases of zoster. Both in human lesions and in the rabbit's cornea, he found acidophilic nuclear inclusion bodies, which he called "zoster bodies." In the rabbit's cornea these bodies were present in greatest numbers on the 4th day after inoculation. The keratitis, when it did develop, was mild, and consisted of small round vesicles, which did not appear till the 4th day, and which after a few days tended to regress. Kundratitz (1924-25) claimed to have transmitted zoster to infants; in contradistinction to herpes febrilis, in which the incubation period of the experimental human disease is only 24 to 48 hours, the incubation period in zoster was 12 to 15 days. Teague and Goodpasture (1923-24) obtained some evidence that the zoster virus differed from that of herpes febrilis mainly in its degree of virulence, but their experiments were not very satisfactory.

appear to have been cut off sharply. Still others assume a highly elongated but oval shape. These last, when short and thick, are often called *coccobacilli*. A given shape is relatively constant within a species although the length-width ratio may vary considerably. The groupings which the cells may assume are a result of post-fission movements, for the plane of division varies rarely if at all. The tendency to remain attached, end to end, resulting in the formation of a chain of *streptobacilli*, is more or less constant for some species but is observed only occasionally in many species. Other groupings of these cells result from the movements of the bacilli after division is complete. Some tend to slide together side by side, a movement known as *slipping*, which results in the formation of palisade-like groups of cells. Still others, in a movement

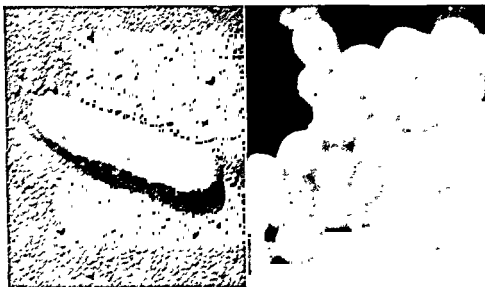


Fig. 3. Electron micrographs of gold-shadow-cast preparations of *Bacillus subtilis* and *Staphylococcus aureus*. The shadow cast preparation illustrates particularly well the occurrence of staphylococci in clusters of attached cells. The cap at the ends of the bacillus and a single flagellum are shown (Lilly Research Laboratories.)

known as *snapping*, bend sharply at the point of division to an acute angle and present a V-shaped appearance. When this type of movement is associated with a tendency for the bacilli to remain attached end to end, chains of organisms resembling a split rail fence may result. Such snapping often results in breakage of a chain of organisms, the parts of which continue to grow in the new direction. The groupings assumed by bacilli are, however, not as characteristic as those of the cocci.

The Spiral Forms. The third principal type of bacterial form commonly observed is that of the spiral or screw-shaped forms. There are a variety of these which may be distinguished morphologically, but at the moment only three main subtypes may be considered. The first of these is the *vibrio* or curved rod. Smears of such organisms often show chains which, since the organisms curve in alternate directions, suggest a spiral organism. It is not difficult, however, to distinguish between such chains and the truly spiral

subsequent observations leave little doubt that they were working, not with varicella virus, but with the so-called Virus III (see p. 2141). On the other hand, the presence of acidophilic intranuclear inclusion bodies in the testicles of vervet monkeys inoculated with material from human chicken-pox lesions was probably the result of infection with the true varicella virus (Rivers 1926, 1927).

Amies (1933) described a specific agglutination reaction, using as antigen a 0.25 per cent. formalized suspension of elementary bodies prepared by the differential centrifugation of human vesicular fluid. Of 55 sera taken from patients 2-12 days after the onset of the attack, 35 contained agglutinins in a titre of 1/4-1/256, and 20 gave negative results. Antibodies appear about 5 days after the onset, and persist for some considerable time. Convalescent serum has apparently no prophylactic value (Lewis *et al.* 1937).

Relation between Herpes zoster and Varicella.

A large body of clinical evidence has been produced suggesting that zoster and varicella are closely allied diseases. The experimental evidence in favour of this conception has been summarized by Lipschütz and Kundratitz (1925) as follows: (1) In infants inoculated with zoster material, apart from the local vesicles that develop, a general varicella-like eruption may occur; (2) children brought into contact with infants inoculated with zoster may develop chicken-pox; (3) inoculation experiments with zoster, made on children who have recovered from varicella, prove negative; (4) inoculation experiments with varicella, in children who have recovered from natural or experimental zoster, prove negative; (5) the serum from patients convalescing from zoster has a protective action against varicella; (6) both varicella and experimental zoster have the same incubation period; (7) similar cytological appearances are found in both diseases.

On the other hand, there are many differences (Barrett 1938). For example, zoster is only slightly contagious: chicken-pox is highly contagious. A leucopenia with a relative mononucleosis is the rule in chicken-pox, but not in zoster. Chicken-pox affords almost complete immunity against chicken-pox, but not against zoster. Zoster affords almost complete immunity against zoster, but not against chicken-pox. Chicken-pox is much commoner in zoster contacts than zoster is in chicken-pox contacts. Zoster occurs as often in persons with, as without, a history of chicken-pox (see Seiler 1949).

The close relationship of the two diseases to each other is supported by the observations of Netter and Urbain (1924, 1926, 1931), who found apparently identical antibodies, demonstrable by the complement-fixation test, in the serum of patients with zoster and varicella. Brain (1933), moreover, obtained equally good fixation with zoster and with varicella vesicular fluids. It has further been shown that serum from convalescent zoster patients often agglutinates a suspension of varicella elementary bodies, though to a rather lower titre than a suspension of zoster elementary bodies. On the other hand, Hasskó, Vámos and Thoroczky (1938) never obtained complete cross-fixation of complement between varicella and zoster sera; and in a high proportion of sera no cross-fixation occurred at all. As already noted, Goodpasture and Anderson (1944) were able to cultivate the zoster virus on pieces of human skin that had been grafted on to the chorio-allantois of the developing chick embryo, but failed to grow the chicken-pox virus under these conditions. The evidence, on the whole, suggests that the two diseases are caused by viruses that are closely related to each other, and possibly even

organisms. A spiral organism which is rigid, or relatively so, is called a *spirillum*, while similar organisms which are flexible are termed *spirochetes*. Further distinctions, such as the presence or absence of an undulating membrane, etc., which are made use of in formal taxonomic schemes, need not concern us here (see p. 728).

Involution Forms. Although the vast majority of bacteria exhibit a marked constancy of form during the early stages of the growth of a culture, in older cultures aberrant forms such as over-sized cocci, Y-shaped bacillary forms and fimbriated forms may be observed. These forms may be the result of aging and represent varying stages in the dis-

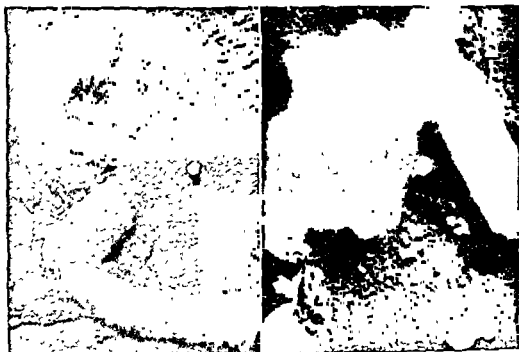


Fig. 4. Electron micrographs of gold-shadow-cast *Bacterium coli* from old cultures. One intact cell is present, and the remainder show the granulation of dead and disintegrating cells. (Lilly Research Laboratories.)

solution of the cell structure. Some workers, however, attach significance to such forms and consider them as indicative of the existence of complex life cycles. Such irregular bizarre forms may often be produced at will by cultivation of bacteria under somewhat adverse conditions of temperature, salt concentration and the like. Morphological variations and their implications are discussed at greater length elsewhere (Chap. 6).

THE STRUCTURE OF BACTERIAL CELLS⁵

The structure of bacterial cells, particularly their finer structure, has been of considerable interest since these organisms were first studied. They are so small, however, that the limitations imposed by the optical system employed become of considerable importance.

⁵ The cytology of bacterial cells is discussed at length by Lewis: *Bact. Rev.*, 1941, 5:181; and by Knaysi: *Elements of Bacterial Cytology*. Comstock Publishing Co., Ithaca, 1944.

endemic, but outbreaks occur from time to time, occasionally reaching epidemic proportions. In the year 1923, for example, 1854 separate outbreaks were reported, necessitating the slaughter of 125,098 animals.

The disease is usually mild, causing a case-fatality rate in cattle of not more than 2-3 per cent., and a slightly higher rate in sheep and pigs; on the Continent, however, a malignant form sometimes occurs, killing 50-70 per cent. of young animals that are attacked. The importance of the disease lies not so much in the deaths to which it gives rise as in the debilitating effect it has on the general health of the animals, leading to a fall in milk and meat production. The incubation period is 2 to 7 days. Infection usually occurs from animal to animal, the infective material coming into contact with the mucosa of the mouth, nose or conjunctiva, or with an abraded skin surface. Cases of direct infection are easy to understand; the real problem lies in explaining the origin of foot-and-mouth disease in animals that have not, so far as can be ascertained, been in contact with infected animals. Numerous explanations have been offered of these cryptogenic cases. Thus it is supposed by some that infection is carried by contaminated hay, straw, milk, drinking water, clothes, and other objects. In support of this, it has been found that the virus may survive on chopped hay at room temperature for at least 15 weeks, and on bran for at least 20 weeks (Report 1927). Moreover, there is evidence that the virus may resist putrefaction for long periods (Report 1928b) and survive in offal (see Henderson and Brooksby 1948). In this way it is possible to understand how infection may lie dormant for months in dried discharges or in carcasses of infected animals. That the virus may not only remain alive, but may keep its virulence under these conditions, was shown by the development of foot-and-mouth disease in a calf fed with hay that had been contaminated 1 month previously with infected saliva, and in pigs fed on crushed bones from the frozen carcasses of infected animals that had been killed several weeks previously (Report 1927).

Another explanation is that the virus is carried by human and animal agencies. Thus it has been suggested: (1) That birds, particularly starlings, may carry infectious material on their feet. For this there is a considerable amount of circumstantial evidence (Bullough 1942, Wilson and Matheson 1952), supported by the fact that large epidemics in England and Wales often coincide with severe disease on the Continent. Direct evidence, however, is lacking, and in the nature of the case would be difficult to secure. (2) That wild rodents may carry the disease and transmit it to cattle, sheep, and pigs coming into contact with them (see Beattie *et al.* 1928). Many of these rodents are susceptible to experimental infection, but practically all experiments designed to test the possibility of natural spread amongst them have given negative results. The virus has, however, been demonstrated in hedgehogs on an infected farm (see McLauchlan and Henderson 1947). Experimentally, it has been found that healthy hedgehogs placed in contact with affected cattle may themselves become infected, and that a healthy cow placed in a stall with infected hedgehogs may develop typical foot-and-mouth disease (Report 1937). (3) That the virus may gain access to, and persist on, the nasopharyngeal mucosa of human beings, who can then act as healthy carriers of the disease; for this there is a certain amount of epidemiological, but no direct experimental evidence (Kling and Hojer 1926). (4) That infected cattle may remain as chronic carriers; it has been found that infective material may persist on the hooves for some time after recovery from the disease, but this appears to be an exceptional occurrence (Gins

The Compound Microscope. With the ordinary microscope objects are viewed by transmitted light and therefore must be sufficiently large to cast a shadow. This size is approximately half the wave length of the light used. The highest numerical aperture that is practical in the objective is 1.4. Such a lens, when used with monochromatic light with a wave length of 5460 Å or 546 m μ , will resolve a particle having a diameter of 0.2 μ . Objects somewhat smaller than this may be seen in that they are visible but are not resolved and neither size nor shape can be determined. Since many bacteria are no wider than 0.2 to 0.5 μ the hope of observing intracellular structures is a faint one. Some workers, Barnard⁶ in particular, have attempted to extend the limits of resolution through the use of ultraviolet light and quartz lenses coupled with "seeing" by means of a photographic plate, but such efforts have not added greatly to knowledge.

The Darkfield or Ultramicroscope. In the instrument known as the darkfield (*Dunkelfeld*) or ultramicroscope, reflected rather than transmitted light is used, objects appearing brilliantly lighted against a black background. The usual microscope may be used as a darkfield by attaching a darkfield condenser in place of the Abbé substage condenser. The principle is the Tyndall effect, perhaps most commonly observed in the appearance of particles of dust in a shaft of sunlight in a darkened room. The limit of resolution is not increased, however, and while exceedingly small objects are visible, they appear only as brilliant points of light. The use of this type of microscope has not contributed materially to knowledge of the structure of the bacterial cell except with regard to the mode of action of flagella. It is, however, very generally used for the detection and observation of very slender microorganisms, especially spirochetes as in exudate from a syphilitic chancre or leptospira in the blood from cases of leptospirosis.

The Electron Microscope.⁷ The recently developed instrument known as the electron microscope operates on a different principle from the optical microscopes in that a stream of electrons is used rather than a beam of light. The electron stream is focused by means of magnetic "lenses," and the interposed object intercepts the stream to cast a "shadow" which is recorded on a photographic plate to give an electron micrograph. Untreated bacteria cast shadows in the electron beam, or the preparation may be "metal shadowed" by the deposition of gold, chromium, etc., at an angle so that the resulting micrograph has a three-dimensional appearance. Remarkable resolution is possible because of the minute size of the electron and sharp photographs may be obtained at magnifications of 30,000 diameters or more. Its application is somewhat limited in that the object must be prepared as a dry film on collodion and observed in a high vacuum, in consequence, living organisms cannot be studied. Application of the electron microscope to biological problems is still in its infancy but has already contributed considerably to knowledge of bacterial cell structure.

Certain structures of bacterial cells are, however, of sufficient magnitude to be seen and studied with the optical microscope. The most obvious of these are structures external to the cell—capsules and flagella.

⁶ Barnard: *Lancet*, 1925, ii 117.

⁷ See Burton and Kohl: *The Electron Microscope Its Fundamental Principles and Applications*. 2nd ed. Reinhold Publishing Corp., New York, 1946.

intramuscular injection of the virus. She found that if one foot was adequately protected from pressure by being wrapped in cotton-wool, no lesions developed on this foot after intramuscular injection, though typical vesicles appeared on the unprotected feet. Moreover, by exposing the feet to unusual pressure, as by keeping the animals in cages with a coarse wire-mesh floor, she was able to induce the formation of vesicles on the toes, and even on the dorsal parts of the feet—situations in which vesicles do not usually occur. The disease can readily be passed from animal to animal by inoculation with infective material. Complement-fixing bodies may be found in the blood serum of convalescent guinea-pigs (see Krag and Schmidt 1937).

Rabbits, rats and mice can all be infected, but the experimental disease is less severe and less typical than in guinea-pigs; dogs and cats sometimes prove susceptible; ducks can be infected by inoculation of the virus into the thickened pads of skin under the toes (Report 1937). Unweaned white mice are highly susceptible to intraperitoneal inoculation, and are even more useful than cattle inoculated into the tongue for the detection of neutralizing antibodies and for revealing antigenic differences between strains belonging to the same type (Skinner *et al.* 1952). The disease practically never spreads from experimentally inoculated to normal rodents. Even highly susceptible cattle placed in contact with infected guinea-pigs rarely contract the disease.

Properties of the Virus.—The virus is present in great concentration in the vesicle fluid of experimentally infected guinea-pigs; even when diluted 1/1,000,000 times it is generally infective for normal guinea-pigs. In the blood, during the febrile stage of the illness, the concentration is less, but even this is active in a 1/1,000 dilution (Report 1927). In cattle during the febrile stage of the disease, the saliva, urine, milk and vesicular fluid are infective; but in 3 to 6 days after the appearance of the lesions they lose their infectivity completely. For diagnostic purposes, it is therefore important to use fresh vesicular material, not more than 2 days old (Olitsky *et al.* 1928). The rapid disappearance of the virus from the local lesion and blood of infected animals is one of the most remarkable features of foot-and-mouth disease; within the space of 3 or 4 days as a rule the virus, from being present in large quantities, is reduced to such an extent that it is no longer demonstrable by experimental methods.

Loeffler and Frosch (1898) in 1897 were the first to show that the foot-and-mouth virus was filtrable. When present in diluted vesicle fluid, it will pass readily through Berkefeld, Chamberland and Seitz filters. The size of the virus is estimated at 8–12 $m\mu$ by Galloway and Elford (1933) using the gradocol membrane technique, at 20–25 $m\mu$ by Elford and Galloway (1937) and Schlesinger and Galloway (1937) using the centrifugal method, and at 40 $m\mu$ by Barnard (1937) using ultra-violet photomicrography. The suggestion that the A, B, and C varieties are not identical in size (see Modrow 1929) finds no support in the observations of Galloway and Elford (1937). The virus was successfully cultivated by Maitland and Maitland (1931) in tissue cultures containing guinea-pig embryo tissue and clotted guinea-pig plasma. Seventeen successive subcultures were observed, and a titre as high as 1/100,000 was obtained (see also Hecke 1930, Striegler 1933, Kôbe and Fertig 1936). More recent methods of culture use foetal calf skin (see Fogedby and Michelsen 1947, Zuloaga 1947), or the mucosa of the bovine tongue (Frenkel 1950, Mace *et al.* 1951). Growth occurs best in a high concentration of oxygen.

The virus is very resistant to certain methods of disinfection, though easily destroyed by others. Thus when vesicle fluid from the guinea-pig is dried rapidly on a glass slide at 37° C., it is often inactivated immediately; but if dried slowly at room temperature, and kept over H_2SO_4 , it survives for 3 to 6 months. When frozen at 30° C. the virus

Capsules. Many bacteria, perhaps potentially all, form a capsule, which apparently originates from the outer layer of the cell membrane; in stained preparations it can sometimes be seen surrounding the cell like a halo. The capsules of bacteria are often one or two times as thick as the diameter of the cell itself and may appear to extend continuously over a chain of organisms or completely surround paired organisms such as diplococci. Cultures of capsulated bacteria are usually slimy and the growth is sticky and viscous. The capsular material is generally polysaccharide in nature, though some bacterial slimes may contain mucin-like proteins. Some of these forms may be a source of considerable annoyance in the sugar refining industries. The presence of a capsule is also associated with the virulence of a variety of pathogenic bacteria. Pneumococci, for example, which have no capsule are relatively avirulent but when capsulated are highly virulent. Anthrax bacilli are almost always found to be capsulated when observed in preparations made from ani-

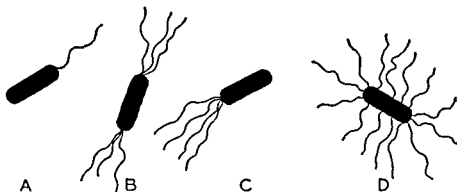


Fig. 5. Position of flagella on the bacterial cell. A, monotrichous; B, amphitrichous; C, lophotrichous; D, peritrichous.

mal tissues. The capsule appears to function as a bacterial defense against the activity of phagocytic cells of the body. The sheaths of some of the filamentous bacteria may possibly be a capsule in somewhat different form. The colorless sulfur bacteria which derive their energy through the oxidation of hydrogen sulfide to elementary sulfur deposit sulfur granules in their sheaths. Likewise the iron bacteria which oxidize iron from the ferrous to the ferric state deposit ferric hydroxide in the sheaths surrounding them.

Flagella. Many kinds of bacteria are observed to be motile under the conditions in which bacteria are usually studied. Some of those forms in which motion has never been observed may possibly possess the power of locomotion under certain unusual conditions. Independent bacterial motion is a true movement of translation, and is to be distinguished from the oscillating or quivering movement (brownian movement) exhibited by all very minute particles suspended in water or other fluids. Many bacteria are found to be motile when they are examined after removal from certain culture media, but non-motile if they have been grown on other substances. The colon bacillus, for example, is motile when picked from young colonies on gelatin or agar but is frequently non-motile when taken from broth. The rate at which a bacterium moves has been approximately measured. The typhoid bacillus may

of neutralizing the virus *in vitro*; these can be titrated with considerable accuracy on the bovine tongue (Brooksby 1949).

Prophylactically, immunity may be produced by the injection of animals with a weakly virulent virus, given either alone or in combination with immune serum, or by a virus inactivated by suitable means. The practice of using living virus is open to several objections. The vaccine that is now used most extensively was developed by Waldmann and his colleagues (Waldmann and Kobe 1938, Waldmann *et al.* 1941-2) by applying the two principles of formol inactivation introduced by Vallée, Carré and Rinjard (1926) and of adsorption on to aluminium hydroxide used by Schmit-Jensen, Schmidt and Hansen (1936). It is made from the vesicle fluid and epithelium of the tongues of cattle that have been infected with a highly virulent strain of virus. The suspension is treated with 0.05 per cent. formalin for 48 hours at 25° C. The virus is so attenuated that it can be injected into healthy cattle without ill effect, though it is doubtful whether it is completely killed (Mohlmann 1952). This vaccine has now been used on a large scale in Europe, Mexico, and South America. Immunity develops in about 10 days. The high cost of the vaccine may be lowered if unweaned mice can be substituted for cattle as a source of virus (see Skinner *et al.* 1952). Other methods of preparing vaccine have been described, such as that in which the blood of infected cattle is treated with 0.05 per cent. crystal violet for 6-8 days at 37° C. (see Galloway *et al.* 1948), or in which virus grown in tissue culture is used (Henderson and Galloway 1953). Vaccination is restricted as a rule to countries in which the disease is endemic. Though it is often of undoubted value in protecting animals against the clinical manifestations of infection, it has several weaknesses. In the first place the immunity conferred is more or less type-specific. So long as the infecting types of virus are similar to those contained in the vaccine, a reasonable degree of protection may be assured; but if a variant type arises or is introduced, then the disease may spread freely in the vaccinated population (see Fluckiger 1953). Again, by modifying an attack of the disease, vaccination may conceal the presence of infection, with the result that the disease may be widely spread by apparently healthy animals. Other objections are that commercial vaccines vary in potency and recommended dosage; that the different variants of the virus may be imperfectly represented in the vaccine; that inactivation may be insufficient so that the vaccine actually spreads the disease, that not all animals react alike to the same vaccine; and that immunity lasts for only about 6 months. Basset (1953), in a critical review, concludes that a policy of vaccination can never be more than a palliative method of combating the disease.

Methods for dealing with an outbreak of foot-and-mouth disease vary in different countries (see Fluckiger 1953). In Great Britain, the United States, and Scandinavia a rigorous policy of stamping out the disease by slaughter and disinfection is practised. In Germany the system known as Ring-Impfung is officially employed. This consists in isolating the affected farms, and giving all contact animals combined active and passive immunization, using for this purpose living virus and polyvalent hyperimmune serum. The result is a mild attack of the disease, which leaves behind it a more or less lasting immunity. At the same time animals on surrounding farms are given an injection of serum, the effect of which is to afford them some degree of passive immunity for about 10 days. The serum may, however, mask a mild attack, so that the infection of some animals passes unrecognized and may form a starting point for further spread. The whole area is isolated for 2-3 weeks.

travel a distance of 4 mm., or about 2000 times its own length, in one hour, the cholera vibrio may attain a speed of 18 cm. per hour for short distances.

This power of locomotion depends upon the possession of flagella, long, fragile, filamentous, coiled appendages. These organs are contractile and their propelling action is due, not to a lashing motion as once thought, but to a rhythmic contraction which moves helicoidally over the surface, the action being that of a screw rather than an oar.⁸ Flagella are very slender, 20 to 50 $m\mu$ in diameter, and their demonstration with the optical microscope commonly requires staining methods, such as silver impregnation or mordanting, by which staining material is deposited upon them to make them thicker than they actually are. It has been reported, however, that flagella may be observed in the living unstained state in some bacteria, such as the very large spirilla. The origin of flagella has been a matter of some interest and it has been generally



Fig. 6. Flagella of bacteria stained and viewed with the optical microscope. (Stained.)

assumed that they are extensions of the cell wall or protoplast.⁹ Knaysi¹⁰ has shown, by means of electron micrographs, that they originate in the protoplasm of the cell, and the more recent evidence of van Iterson and Robinow, illustrated in Fig. 7, indicates that their origin is in small spherical bodies lying between the cell wall and cytoplasmic membrane. Whether these correspond to the blepharoplasts of flagellated protozoa is not yet clear. It may be noted that the suggestion of Pijper¹¹ that flagella are capsular polysaccharide twisted off in threads by the motion of the cells, and therefore artifacts, has no basis in substantial evidence.

Bacteria differ from one another with respect to the position of the flagella on the cell body. The *monotrichous* organisms have only a single flagellum at one end, the *amphitrichous* bacteria have either a single flagellum or a tuft

⁸ Pijper. Jour. Path. Bact., 1938, 47:1.

⁹ See the review by Knaysi: Bot. Rev., 1938, 4:86, 99.

¹⁰ Knaysi. Science, 1942, 95:406.

¹¹ Pijper. Jour. Biol. Photo Assn., 1947, 16:3.

(1936). Outbreaks occurred irregularly, but on a generally increasing scale, till in 1939-40 the number of infected premises was 123 and the number of animals on these premises was over 220,000 (White 1940). It is an acute febrile disease, characterized by vesicles on the snout, lips, oral mucosa, tongue, and feet. The vesicles are 2-3 mm. and upwards in diameter. There is often considerable systemic disturbance, the temperature reaching 108° F. The animals suffer from varying degrees of lameness. Growth is seriously interrupted. The case fatality is low except in young sucking pigs, which may die either as the result of cessation of lactation in the nursing sow or of mechanical obstruction to respiration by the vesicles. Animals that recover develop a temporary immunity, which passes off in a few months' time. The disease spreads readily by contact. The incubation period is about 48 hours. Experimental inoculation is successful by the intradermal or intramuscular route. Clinically, the disease cannot be distinguished with certainty from foot-and-mouth disease, and reliance in the first place has to rest on animal inoculation or on the complement-fixation test. As will be seen from Table 176 the virus of vesicular exanthema of swine differs from that of foot-and-mouth disease in being pathogenic to horses, but not to cattle or guinea-pigs

TABLE 176

DIFFERENTIAL SUSCEPTIBILITY OF ANIMALS TO INOCULATION (Traum 1936)

Virus.	Swine	Cattle.	Guinea-pigs	Horses
Foot-and-mouth . . .	+	+	+	-
Vesicular stomatitis . .	+	+	+	+
Vesicular exanthema . .	+	-	-	±

+ = susceptibility. ± = susceptibility less and irregular. - = insusceptibility

The susceptibility of the horse to vesicular exanthema is less than that of cattle, and may perhaps vary with different strains of virus. The British workers (Report 1937) failed in their efforts to infect horses with a strain of Californian origin. The virus is antigenically different from any of the known types of foot-and-mouth virus, and there appears to be no cross-immunization between the two viruses. Three different serological types are recognized. The disease is spread largely by the practice of feeding swine on raw garbage. In control of the disease reliance is placed on strict quarantine measures.

LUMPY SKIN DISEASE OF CATTLE

This disease, sometimes known as pseudo-urticaria, is common among dairy herds in the Cape peninsula of South Africa. It is characterized by transient, nodular lesions in the skin, and enlargement with subsequent necrosis of the superficial lymphatic nodes. The case fatality is low, but the condition and the milk yield falls. Inclusion bodies staining found in the cytoplasm of the epithelial cells (de Kock 1941). Don and Kipps (1949) described a fatal case in a calf using chick embryos presented a change in feather formation. The virus survives in 50 per cent. glycerol.

of flagella at each end; the *lophotrichous* bacteria have a tuft of flagella at one end; and the *peritrichous* have flagella projecting from the whole body of the cell, the sides as well as the ends. Bacteria having no flagella are termed *atrichous*.

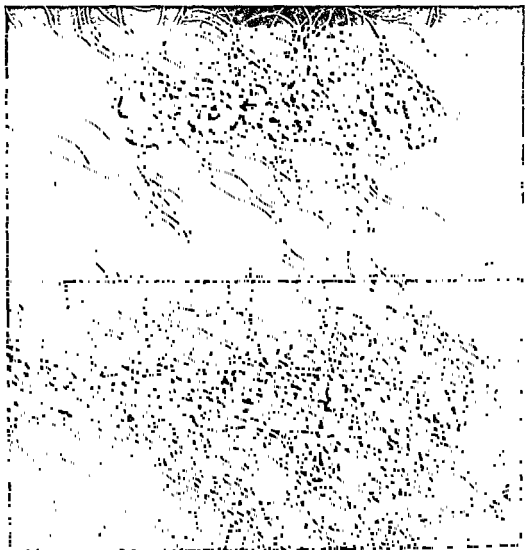


Fig 7. Electron micrographs of shadow-cast preparations showing the flagella of *Proteus vulgaris*. The bacteria were grown to the swarming stage on agar, refrigerated at 5° C. for 16 to 20 hours, floated off in 5 per cent formalin and washed twice before mounting on collodion. The preparations are very transparent and flatten completely so that certain of the internal structures are demonstrable by shadowing. The origin of the flagella in small, spherical bodies about 100 m μ in diameter and remarkably uniform in size, situated between the cell wall and cytoplasmic membrane, is clearly shown (From electron micrographs by Woutera van Iterson and C. F. Robinow. A full account of this work will appear in *Biochimica et Biophysica Acta*.)

The number of flagella on the peritrichous bacteria varies considerably and even closely allied species may differ from one another in this respect. The typhoid bacillus, for example, has, as a rule, more flagella (ten to twelve) than the colon bacillus (two to six). The majority of actively motile bacteria belong either to the bacilli or spirilla, very few cocci are motile under ordinary

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conditions. Within a given bacterial species different degrees of motility may be found. Some strains within a species may consist practically entirely of motile cells, while other strains may show no motility in hanging drop preparations nor may flagella be found in stained smears. Further, the motility of some strains is affected by the temperature of incubation while that of others is not, some strains may be motile when grown at 22° C. but not when grown at 37° C. Motility, therefore, is not a character that can be used for exact delimitation of species or varieties of bacteria. Standard cultural characters and biochemical reactions do not appear to be correlated with the presence or absence of motility.

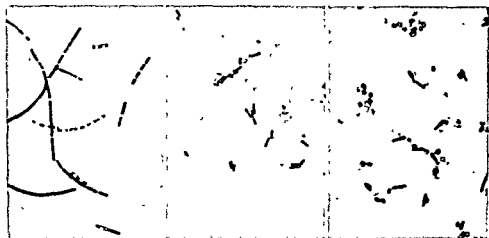


Fig. 8 Spore formation by bacteria. Left, the anthrax bacillus which forms central spores which do not distend the wall of the vegetative cell, the tendency of the bacilli to occur in chains is also characteristic. Center, *Clostridium botulinum* type B, which shows the typical clostridial form with terminal spore of greater diameter than the vegetative cell, note the free spores separated from disintegrated vegetative cells. Right, *Clostridium sporogenes*, showing a subterminal clostridial spore. These preparations are all stained with a single stain in the usual way, the vegetative cells take up the dye but the spores remain unstained Fuchsin, $\times 1050$.

Spores.¹² Some of the bacilli possess the ability to form resistant structures known as spores. Such spores, spherical or oval in shape, show a relatively high resistance to all sorts of injurious influences, including high temperatures, germicidal chemicals, etc. Furthermore, they stain with great difficulty. Usually heat in combination with a mordant is necessary to drive the dye into them.

An assembling or concentration of nuclear material (i.e., material staining deeply with basic dyes) precedes spore formation in some cases and constitutes the spore primordium. Cytological studies suggest that in some bacteria a granule forms which enlarges to become the spore, in others there is an aggregation of granules, and in still others no granules are formed and what appears to be a condensation of the protoplasm occurs. The highly refractive character of the unstained spore and its reduced water content probably are connected with the concentrated character of the spore substance. Such spores

¹² See the review by Knaysi: Bact. Rev., 1948, 12 19.

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may be formed in any part of the cell, the position generally being relatively constant within a species. The spore is spoken of as *terminal* if formed at one end of the cell, *central* if formed in the center of the cell, and *subterminal* if formed half way between the center and the end of the vegetative cell. In some cases the spore does not exceed the diameter of the parent cell, as in the anthrax bacillus, in others where its diameter is greater than that of the vegetative cell it causes a bulging out of the wall of the cell at the point where it lies. If such spores are terminal, a drum-stick appearance may result, as in the tetanus bacillus; or if it is central, the vegetative cell becomes spindle-shaped. In simple stains the spore appears as an unstained round or oval body lying within the stained protoplasm of the vegetative cell. When the spore is fully developed, the vegetative cell disintegrates and disappears.



Fig. 9
spore ca
to end
hydrochloric acid fixation, Giemsa, $\times 4000$. (Robinow.)

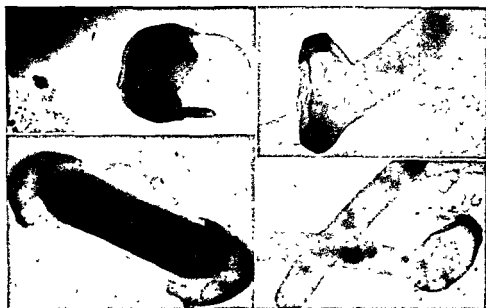
The conditions under which bacteria form spores vary with the nature of the organism. The anthrax bacillus, an aerobe, forms spores only when in contact with free oxygen, a fact that has practical bearing on the disposal of bodies of cattle dead of the disease. The tetanus bacillus, on the other hand, and the anaerobes in general, form spores in the entire absence of oxygen. A suitable temperature is essential to the formation of spores. The anthrax bacillus forms spores most abundantly at 30°C to 32°C , and will not produce them at a temperature of 12°C . Lack of food is apparently not an adequate stimulus to spore formation. In all cases a period of uninterrupted vegetative multiplication precedes the appearance of spores, and the conditions necessary for their production seem to arrive simultaneously for most of the cells in a culture. It has been suggested that diminution in the water content of the bacterial cell, leading to a shrinking of the colloids, is the main factor in bringing about spore formation. Friedman and Henry¹³ have found that the percentage of bound water is relatively high in spores as compared with

¹³ Friedman and Henry Jour. Bact., 1938, 36 99.

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vegetative cells, and suggest that this is associated with their heat resistance. In any case, spores are not formed under adverse conditions, as it might be thought, but in circumstances favorable to growth.

When spores are brought under conditions favorable for growth they *germinate* and become vegetative cells which multiply in the usual manner. The rate of germination has been found to be logarithmic¹⁴ but delayed germination occurs with some frequency and in some cases appears to be due to the presence of inhibiting substances, such as oleic and linoleic acids, in the culture medium rather than a delayed response to a favorable environment.¹⁵ As it undergoes germination the spore shows a change in its refractive properties, probably due to the imbibition of water. The entire spore wall may



become thin and stretch and the spore assume a bacillary form, or a vegetative cell may burst through the spore membrane, the empty case being cast off as a hull. The anthrax bacillus grows out of one side of the spore wall; the closely related hay bacillus grows out of opposite sides simultaneously. Other forms of bacteria exhibit intermediate methods of germination. Also, irregularities may occur in the development of spores of the same species. The process of development of the vegetative cell and its escape from the spore case is particularly well illustrated in the electron micrographs in Fig. 10, taken by Knaysi who has studied the process of sporulation in detail.¹⁶

¹⁴ Wynne and Foster Jour. Bact. 1948, 55 69.

¹⁵ See the general discussion of studies on this point by Foster and Wynne Jour. Bact., 1948, 55 623.

¹⁶ Knaysi: Jour. Bact., 1945, 49, 473, 617, *ibid*, 1946, 51 187. *ibid*, 1947, 53 525.

the tentative conclusions sponsored by different observers. One reason for this is that the point at issue has not always been clearly defined. Sometimes the problem actually submitted to experimental study has been the correlation between resistance to a particular bacterium and the presence of the corresponding antibodies in the circulating blood. But we shall see, when studying anaphylaxis, that specific antibodies readily become fixed to tissue cells; so that the demonstration of immunity in the absence of circulating antibodies, or a failure to find any close association between antibody titre and degree of resistance, cannot be accepted as satisfactory evidence that specific antibodies are not involved.

As an example of the relations that hold in experiments of this kind we may refer again to the studies of Wright (1927).

In these experiments, a large series of rabbits were immunized by a single intravenous inoculation of killed culture and were tested at various intervals thereafter by determining the rate at which virulent pneumococci disappeared from the blood stream, after the intravenous injection of 1 ml. of living culture. The results suggested that there was a slight, but definite, improvement in the clearing capacity within a few hours. This became well established on the 3rd day, was still marked at the end of a month, and might persist for 3 to 6 months, though in diminishing degree. The serum of many of these rabbits was tested for the presence of agglutinins, opsonins, and precipitins. It was found that the production of these antibodies was inconstant, and that the times of their appearance and disappearance in the blood in no way corresponded to the duration of the improved clearing capacity. In many cases no antibodies were ever demonstrable, although the rabbit showed a well-marked clearing reaction. In others, agglutinins appeared to low titre on the 4th day, increased in amount to the 7th or 10th day, and disappeared by the end of the 3rd week. Thus they first appeared after the increased clearing capacity had become well established, and vanished while it was still present in a marked degree. Wright also determined the inhibiting action of whole blood (heparinized) on the growth of pneumococci, using a modification of the methods employed by earlier workers in determining the bactericidal action of whole blood on this organism. He found that the results of this *in vitro* test were far more closely correlated with the clearing capacity of the rabbits, and with the power of their serum to confer this clearing capacity on normal rabbits, than were the results obtained by agglutination or precipitin reactions.

A protocol, taken from his paper, and slightly modified in form, affords a clear illustration of the general type of his results. (Table 71.)

The lack of correspondence between the presence of agglutinins and precipitins on the one hand, and clearing capacity on the other, is obvious; but the association between the growth-inhibitory action of the whole blood and the clearing capacity is very close. Similarly, those rabbits which are able to remove pneumococci rapidly and completely from their own circulating blood are in a position to confer a similar ability on normal rabbits, by the transference to the latter of a relatively small proportion of their blood serum. The correspondence, in this case, is not absolute; but the association is well-marked.

It should, perhaps, be noted that other workers have reported the occurrence of an effective active immunity in the entire absence of circulating antibodies, as judged by all available tests, including the bactericidal power of the whole blood (see, for instance, Teale 1935).

As we relate in Chapter 50, antibody may be formed locally in the connective tissue, by cells proliferating there in response to the antigenic stimulus. It is therefore possible that the immune response of the infecting organism in the connective tissue may be a locally effective response; but Nunes (1950) reports substantial amounts of agglutinin in the blood of guinea-pigs within 5 hours of an intraperitoneal injection of a massive infecting dose of pneumococci.

Spore formation is not very common among bacteria. It has been definitely observed only in the rod forms; cocci are not known to sporulate, and sporulating spirilla, if they exist, are rare. The majority of the obligate anaerobes produce spores, as do some of the aerobic bacilli. The spore-forming bacteria of known pathogenicity for man are few, a fortunate circumstance that materially facilitates and simplifies disinfection and the treatment of infectious disease. Physiologically, the spore is usually considered as a resting stage, serving to tide the species over unfavorable periods. From this point of view, the spore stage is analogous to the periods of hibernation or estivation among higher



Fig. 11. Electron micrograph of *Clostridium tetani* from 24 hour culture. The protoplasm is homogeneous at this stage and the clear cell walls are apparent. Peritrichous flagella are shown also, and the bacilli are in various stages of cell division. Compare the latter with Fig. 15. (Mudd and Anderson, SAB No. 61.)

forms of life and the living matter of the spore may remain dormant for years or even decades. It does represent a definite differentiation of the vegetative cell as indicated by the occurrence of antigens in the spore that differ from those of the vegetative cell as well as antigens shared by the two forms.¹⁷ Spore formation is not a form of multiplication, for each vegetative cell forms only a single spore and each spore germinates into a single vegetative cell. Obviously no increase in numbers is possible by this process.

The Finer Structure of the Bacterial Cell. Knowledge of the intracellular structures of bacteria is strictly limited, as pointed out above, by limits in the resolving powers of the microscope. Three questions have been of interest, namely: the existence and nature of the cell membrane, the nature of the cell substance or entoplasm, and, finally, the question of the existence of a nucleus.

¹⁷ See Doak and Lamanna: Jour. Bact., 1948, 55 369.

HUMAN ENCEPHALITIS

Encephalitis Lethargica.

This is a primary encephalitis, presumably of virus origin. The disease was first described by von Economo (1917) in Vienna, and was recognized in this country the following year. The highest year of incidence in England and Wales was 1924, when 5,039 cases were notified with 1,407 deaths. Since then the incidence has declined gradually, and by 1950 the number of registered deaths had fallen to 115. From post-mortem observations, however, it appears very doubtful whether more than a small proportion of the cases diagnosed as encephalitis lethargica are really examples of this disease. The true incidence is probably far lower than is indicated by the reported figures. *Epidemic hiccup* is regarded as a mild form of the disease (see MacNalty 1937).

Conflicting reports have been published on the aetiology of encephalitis lethargica. Much of the earlier work on the transmission of infection to animals was confused by the occurrence of a spontaneous disease of rabbits—meningo-encephalitis (see p. 2161)—and must be disregarded. Observations by Levaditi and Harvier (1922-23), Perdrau (1925*a, b*), da Fano and Perdrau (1927), and Gay and Holden (1933) suggest that the virus of encephalitis lethargica may be related to that of herpes, but this is little more than speculation. Though both McIntosh (1923) and McCartney (1924*b*) have reported the transmission of a fatal encephalitis to rabbits by the direct inoculation of brain suspensions from infected patients, it must be admitted that very little progress has been made in the study of this disease and its causation still remains obscure. (For a short summary of the experimental observations made on this disease the reader is referred to pages 1499-1501 in the 2nd edition of this book.)

The Arthropod-borne Group

This comprises a number of diseases conveyed, usually by mosquitoes, to man and sometimes the larger animals from a reservoir which may be in small mammals and birds or in insects. Many of the causative viruses are closely related to each other, and many form a haemagglutinin acting on the red blood corpuscles of sheep, pigeons, or newly hatched chicks (see Macdonald 1952, Chanock and Sabin 1953).

St. Louis Encephalitis.—A very remarkable outbreak of encephalitis occurred in St. Louis, Mo., during the late summer of 1933. The epidemic started at the beginning of August and finished about the end of October. Rather over a thousand persons were attacked—an incidence of approximately 1 per 1,000 of the population. The case-fatality rate, which had an average of 20 per cent., showed a striking increase in relation to the age of the patient, thus, under 40 years of age it was less than 10 per cent.; in the 40-50 age group it was 12 per cent., in the 50-60 group 21 per cent., in the 60-70 group 38 per cent., in the 70-80 group 56 per cent., and in the 80-90 group 80 per cent. Over half the fatal cases died in the 1st week. The incubation period was not determined with certainty, but appeared to be from 4 to 21 days. Pathologically, the lesions differed in several respects from those in encephalitis lethargica, particularly in the more extensive involvement of the meninges and spinal cord, the more widespread distribution of inflammatory foci through the brain, and the greater frequency of degenerative changes in the nerve cells and of neuronophagia (Report 1935).

Cultures of the blood and spinal fluid were sterile. Attempts to transmit the

The cell membrane of bacteria is not demonstrable as a structurally differentiated envelope, as that of many plant cells is, by immersion of the cells in hypertonic solutions. Robinow has found that treatment with boiling dilute sodium hydroxide results in a shrinkage of the protoplasm away from the cell wall, allowing its direct demonstration by staining with crystal violet. Such rigorous treatment may, of course, result in artifacts but a cell wall appears clearly in electron micrographs as shown in Fig. 11, and studies by Mudd and his associates¹⁸ with the electron microscope have shown that it is a definite morphological structure from which the cytoplasm may shrink away and which is sufficiently solid to show jagged lines of fracture when broken by sonic vibrations. It would appear to have considerable rigidity, particularly among the bacilli, in order to maintain shapes other than spherical. Experi-

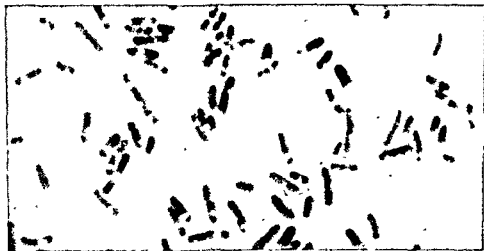


Fig. 12. Irregular and bipolar staining of the plague bacillus. Note the heavily stained areas and metachromatic granules. Fixed in methyl alcohol and stained with methylene blue, $\times 2400$.

ments on the growth of the colon bacillus in media in which the surface tension had been reduced by the addition of sodium oleate resulted in the production of long, filamentous forms, much longer than the usual rods. Such experiments have been interpreted as indicating that this organism is essentially tubular, the sides having sufficient rigidity to withstand the reduced surface tension but the length increasing because of a less rigid structure at the ends.

Knaysi¹⁹ has found that the cell wall of *Bacillus cereus* and *Bacillus megaterium* is made up of lipid and protein in stable combination and is much more resistant to autolysis than the cytoplasm. It stains with dyes of the Sudan series, and he has found that the inner surface is jagged and irregular, tending to divide the cell into compartments. It is possibly this last that results in the lack of a sharply defined interface between the entoplasm and the outer cell wall in the usual preparations. The precise nature of the bacterial cell membrane is of particular interest in connection with the problems of permeability.

¹⁸ Mudd *et al.*: Jour. Bact. 1941, 41:415, *ibid.*, 1941, 42:251.

¹⁹ Knaysi. Jour. Bact., 1946, 51:113.

and his colleagues (1942*b*, *c*) isolated three strains of the St. Louis virus from *Culex tarsalis* Coquillett in the Yakima Valley and proved these insects to be capable of transmitting infection under experimental conditions. The real reservoir of infection, however, appears to be the mite, which infects chickens and—probably more important—wild birds in their nests. These birds develop a viremia which enables the mosquitoes to become infected and so transmit the disease to man (see Hammon *et al.* 1951). *Diagnosis* of the disease during life is aided by the complement-fixation test, which is usually positive within the first two weeks. Neutralizing antibodies appear later, but are less liable to give false positive reactions. At autopsy the organism may be isolated from the central nervous system by mouse inoculation.

Japanese B Encephalitis.—SYNONYM: *B encephalitis*; *Russian autumn encephalitis*.—In Japan several outbreaks of encephalitis have occurred during the past 100 years or so. They have been particularly common during the late summer months, and for this reason the disease is often referred to as “summer encephalitis.” A large outbreak occurred in the summer and autumn of 1924, when there were over 7,000 cases with a fatality rate of 60 per cent. Another large epidemic, causing about 5,000 deaths, was witnessed in August and September 1935. The American troops suffered in Okinawa (see Tigertt *et al.* 1950); and there was a severe outbreak in Korea in August 1949, lasting for about 6 weeks, with 5,548 reported cases and 2,429 deaths (Hullinghorst *et al.* 1951). The disease is said to be endemic in Malaya (Paterson *et al.* 1952), and possibly in the Sudan and Congo (Smithburn and Jacobs 1942). The incubation period is 8–15 days, most often 11–12 (Mosher 1947). Pathologically the disease is a meningoencephalitis, characterized by widespread cortical damage with ganglion cell degeneration, focal perivascular lymphocytic infiltration, destruction of the Purkinje cells of the cerebellum, and changes in the cord almost identical with those of poliomyelitis. Judged by the presence of neutralizing antibodies, inapparent infections are very common in the normal population, and in domestic animals such as the horse, cow, pig, and goat (Bawell *et al.* 1950).

Horses may also suffer from overt disease. Burns, Tigertt and Matumoto (1949) described an outbreak of encephalomyelitis in these animals, and isolated 2 strains of virus that were immunologically similar to, if not identical with, the virus responsible or infecting man.

For a general review of Japanese B encephalitis, see Warren (1946), Olitsky and Casals (1952).

The causative virus was isolated and studied by Kasahara and his colleagues (1936*a*, *b*, 1937*a*, *b*) and by Taniguchi and his colleagues (1936). It is about 20–30 μ in size (Yaoi *et al.* 1939); it can be cultivated in a medium of the Maitland type containing minced chick embryo brain (Kawakita 1939); it is avirulent to rabbits, but gives rise to a non-suppurative meningo-encephalomyelitis on intracerebral inoculation of mice, monkeys, and young sheep; it is neutralized by the serum of convalescent patients; and it can be distinguished by cross-neutralization tests from the virus of St. Louis encephalitis (Kawamura *et al.* 1936, Kasahara *et al.* 1937*a*, Webster 1938*a*) and from the West Nile virus (Casals 1944). Guinea-pigs are said by the Japanese workers (Kasahara *et al.* 1936*a*) to be susceptible to intratesticular inoculation, but they appear to be resistant to intracerebral inoculation (Webster 1938*a*). Albino rats 7 days old can be

The nature of the cell substance is not well known. It is, of course, protoplasm, as the ancillary evidence of chemical composition indicates. The cells of actively growing cultures appear to be homogeneous both in the unstained state and when stained by various dyes. As the culture becomes older, however, the cells of many organisms may be found to contain granular material which is somewhat more refractive than the surrounding protoplasm in the unstained state, and which takes up certain dyes with greater avidity than the remainder of the cell. These granules are called *metachromatic granules* or, after the workers who first described them, *Babes-Ernst granules*. They are sometimes scattered through the cell substance, and sometimes massed at either end, where they constitute the "polar granules" observed in the plague bacillus, the glanders bacillus and certain other bacteria. In certain species



Fig. 13. Metachromatic granules and bipolar staining in the diphtheria bacillus. Note the differences between these organisms and the plague bacilli. Methylene blue, $\times 1975$.

the metachromatic granules are particularly easy to demonstrate, and their abundance may even constitute a character of some differential value. The nature of these granules has been the subject of microchemical investigations, but the results are somewhat of polysaccharide material of designated *granulose*.

appear to consist of nucleoprotein and take up the nuclear stains with great avidity. This nucleoprotein has been termed *volutin*, and granules of this and other material are generally spoken of as "reserve material"—a term which, because of its implications, could hardly have been less wisely chosen.

The apparent lack of a discrete nucleus in the cells of the true bacteria has been a source of much speculation. Granular materials such as volutin and metachromatic granules which are characterized by staining deeply with basic dyes are sometimes referred to as "nucleoids" but apparently have no nuclear function. The seeming homogeneity of the cell substance, both in the stained and unstained states, has been interpreted by some to indicate that these cells

and his colleagues (1942b, c) isolated three strains of the St. Louis virus from *Culex tarsalis* Coquillett in the Yakima Valley and proved these insects to be capable of transmitting infection under experimental conditions. The real reservoir of infection, however, appears to be the mite, which infects chickens and—probably more important—wild birds in their nests. These birds develop a viremia which enables the

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The causative virus was isolated and studied by Kasahara and his colleagues (1936a, b, 1937a, b) and by Taniguchi and his colleagues (1936). It is about 20–30 $m\mu$ in size (Yaoi *et al.* 1939); it can be cultivated in a medium of the Maitland type containing minced chick embryo brain (Kawakita 1939); it is avirulent to rabbits, but gives rise to a non-suppurative meningo-encephalomyelitis on intracerebral inoculation of mice, monkeys, and young sheep; it is neutralized by the serum of convalescent patients; and it can be distinguished by cross-neutralization tests from the virus of St. Louis encephalitis (Kawamura *et al.* 1936, Kasahara *et al.* 1937a, Webster 1938a) and from the West Nile virus (Casals 1944). Guinea-pigs are said by the Japanese workers (Kasahara *et al.* 1936a) to be susceptible to intratesticular inoculation, but they appear to be resistant to intracerebral inoculation (Webster 1938a). Albino rats 7 days old can be

consist entirely of cytoplasm and have no nucleus, and by others to mean that these cells are all nucleus and have no cytoplasm.

There is no doubt, however, that the bacterial cell contains relatively enormous amounts of nucleoprotein and nucleic acids demonstrable by microchemical methods such as the Fuelgen reaction, and the content of purine and pyrimidine nitrogen found by analysis is very high. It is clear that nuclear material is present in more than adequate amounts, and it has been customary to assume that the bacterial cell contains the chemical equivalent of a nucleus even though no morphologically differentiable structure could be demonstrated. More recently Robinow²⁰ has shown that the very faintly discernible areas apparent in bacteria stained with polychrome stains may be clearly shown by fixation with osmium tetroxide, differentiation in hot hydrochloric acid, and staining with Giemsa. He has called these chromatinic bodies, and their normal occurrence has received strong support from the studies of Hillier, Mudd and Smith²¹ who have found them in electron micrographs of preparations receiving no treatment other than drying. These chromatinic bodies are illustrated in both light and electron micrographs in Fig. 14. They show a strong morphological resemblance to nuclei, stain like nuclei with Giemsa, and appear to divide in the growing cell just prior to fission. Whether they represent true nuclei, analogous to those of other cells, remains to be fully established, but the implication that they are seems very strong.

COLONIAL MORPHOLOGY

A single bacterial cell or group of cells, when planted on a semisolid medium and allowed to develop under suitable conditions of moisture, temperature and air supply, will, in a few hours or days, develop a "colony" so large that it can be plainly seen with the naked eye. In many instances such masses of cells, particularly when the growth occurs on certain culture media, possess salient peculiarities which are characteristic of the species. Growths upon nutrient gelatin are especially characteristic, but upon nutrient agar the morphology of bacterial colonies is less distinctive. Colonies on potato and other solid food substances are, as a rule, still less characteristic. As was pointed out previously, colonial differences may often be accentuated by growth on media containing dyes and other compounds. Diphtheria bacilli, for example, form highly characteristic colonies on media containing potassium tellurate. The character of colonies is also somewhat affected by the density and viscosity of the culture medium and by the physical conditions under which the organisms develop. In general, however, the colony characteristics of a bacterial species are distinctive and are an aid in the isolation and identification of the organism. These characteristics include the size of the colony, its form—whether raised, flat, etc.—and the shape of its edges; its consistency and texture; its surface—whether roughened or smooth; its color, and other similar peculiarities.

These colony characteristics in many cases have their roots in the mor-

²⁰ Robinow. Jour. Hyg., 1944, 43, 413.

²¹ Hillier, Mudd and Smith. Jour. Bact., in press, personal communication from Dr. Mudd.

the brain after death. It is 15-25 μ in diameter. It grows on the chorio-allantoic membrane of the developing chick embryo. It is pathogenic to mice on subcutaneous as well as on intracerebral inoculation. It is virulent for sheep, moderately virulent for rhesus monkeys, and non-virulent for guinea-pigs and rabbits. The disease occurs in the spring and summer months, and is transmitted by the bite of the tick, *Ixodes persulcatus* Schulz. Numerous wild animals and birds are susceptible to experimental inoculation, and natural infection has been found in some rodents. It seems probable, however, that the tick constitutes the natural reservoir, since infection is transmitted by the ovarian route from generation to generation. The disease leaves behind it a lasting immunity. Neutralizing antibodies are demonstrable in the patient's blood about the end of the 2nd week but do not reach their maximum for 2-3 months.

Apart from the Far Eastern type, there is a Western type of spring-summer encephalitis that appears to occur not only in western Russia but also in Czechoslovakia (Krejci 1949, Rampas and Gallia 1949). It is a milder disease and has a lower case-fatality rate. Whether the viruses responsible for these two types are identical with each other is still doubtful, but both of them are very closely related to the virus of louping-ill. Pond, Russ and Warren (1953), who compared strains of Russian and Czechoslovakian encephalitis viruses with the louping-ill virus, concluded that, though there were minor differences between them, they must all be regarded as belonging to a single virus. For a general review of Russian spring-summer encephalitis, see Warren (1946), Olitsky and Casals (1952).

Encephalomyelitis due to Equine virus.—As its name implies, equine encephalomyelitis is essentially a disease of horses (see below). During the late summer and early autumn of 1938 there was a large outbreak in the United States, and human cases of the disease were recognized for the first time. An even larger outbreak affecting nearly 3000 persons occurred in 1941, mainly in North and South Dakota and Minnesota. Diagnosis of infection with the eastern type of virus was made by Fothergill, Dingle, Farber and Connerley (1938) and by Webster and Wright (1938), and with the western type of virus by Howitt (1938b). The disease occurred in rural areas, and affected particularly infants and young children, in whom it caused a high mortality. The virus can sometimes be demonstrated in the blood (Howitt 1939) and in the cerebrospinal fluid (Fothergill, Holden and Wyckoff 1939) during life. After death it may be isolated from the brain by intracerebral inoculation of mice. Neutralizing antibodies are often present in the blood serum and the cerebrospinal fluid of convalescent patients (Howitt 1941, Buss and Howitt 1941). Healthy contacts who have been exposed to infection may also have neutralizing antibodies (Mitchell and Pullin 1943). Diagnosis during life may be made by the demonstration of complement-fixing and neutralizing antibodies in the patient's serum (Casals and Palacios 1941, Casals 1941, Howitt 1943). Infection is probably carried by mosquitoes—*Culex tarsalis* Coquillett (Hammon *et al.* 1942a, b). Besides the disease due to the eastern and western types of virus, laboratory infections have been described with the Venezuelan type (Casals *et al.* 1943).

Encephalitis due to other Viruses.—Several other arthropod-borne viruses are known that cause or are suspected to cause encephalitis. Three of these—the West Nile, the Bwamba, and the Mengo viruses—were isolated from human cases in East Africa; others, such as the Semliki Forest, the Bunyamwera, the Ntaya, the Zika and the Uganda viruses in East Africa, the California virus in the United States, the Ilheus virus in Brazil, and the Anopheles A and B and Wyeomyia



Fig. 14. The chromatinic bodies of *Bacterium coli* which are interpreted as representing the bacterial nuclear apparatus. The upper section of the plate is an electron micrograph of an unfixed preparation from a 3 hour culture of *Bact. coli*. The light areas within the bacterial protoplasm correspond with those faintly discernible with the light microscope and clearly discernible after fixation with osmium tetroxide vapor. (Hillier, Mudd and Smith,

viruses in Colombia, were isolated from mosquitoes. They were detected by virtue of their neurotropic properties for mice. Surveys in Uganda and Tanganyika showed the presence of neutralizing antibodies in some samples of human serum against the Bwamba, the Ntaya, the Zika, and occasionally the Mengo virus, indicating that these viruses were able to infect man (Smithburn 1952); and three cases of febrile disease associated with the Zika virus have since been described (Macnamara 1951). Antibodies were also found to the Ilhéus virus in natives of Brazil (Southam and Moore 1951). Whether, and if so how often, these viruses infect the central nervous system of man is so far undetermined, though human volunteer experiments have shown that the Ilhéus and the Bunyamwera viruses are able to cause encephalitis (Southam and Moore 1951). According to their size estimated by filtration they fall into 3 groups: (1) Anopheles A and B, Wyeomyia, Ntaya, Bwamba fever, and Bunyamwera viruses ranging from 70-122 $m\mu$ in diameter; (2) West Nile, Semliki Forest, Kumbia, Zika, Ilhéus, and Uganda S viruses of 18-30 $m\mu$; and (3) Mengo virus of 10-15 $m\mu$ (Smithburn and Bugher 1953). We append short notes on some of these viruses.

West Nile virus.—This virus was isolated in 1937 from the blood of an African woman suffering from a mild febrile disease. Neutralizing antibodies were subsequently demonstrated in her serum (Smithburn *et al.* 1940). The virus is pathogenic for mice and monkeys injected intracerebrally, and produces in the Purkinje cells of the cerebellum degenerative changes similar to those found in Japanese B encephalitis and in louping-ill. It is estimated to be 21-31 $m\mu$ in diameter. Immunologically it is closely related to the St. Louis and the Japanese B encephalitis viruses (Smithburn 1942). There is evidence that the virus is widespread in Egypt and Equatorial Africa. Goldblum, Sterk and Paderski (1954), who isolated it from 9 cases in Israel, found that the disease to which it gave rise was characterized by an incubation period of 2-6 days, a sudden onset with fever, drowsiness, frontal headache, aching of the eyes on movement, and general enlargement of the lymph nodes. The illness lasted 3-6 days, but convalescence was slow and the lymph nodes took 1-2 months to go down. A rise in the titre of complement-fixing antibodies was demonstrated. According to Smithburn (1954), the West Nile and Ntaya viruses are related to each other and to the virus of Japanese B encephalitis.

Bwamba Fever.—A mild non-fatal disease, caused by a virus producing nervous symptoms in mice, was encountered in African natives by Smithburn, Mahaffy and Paul (1941) during a yellow fever survey in the Western Province of Uganda and was named by them Bwamba fever. Clinically, the disease was characterized by a sudden onset with fever, headache, and pain in the lumbar region, mild conjunctival injection and a skin rash. The fever lasted for 2-5 days, the headache and backache for 2 or 3 days longer. Convalescence was rapid and complete. The disease did not appear to be highly contagious. A virus could be demonstrated in the blood by intracerebral inoculation of mice, and could be carried over from one mouse to another by injection of brain tissue. After intracerebral injection symptoms of nervous excitement appeared in about 4 days, and the animals generally died a day later. At post-mortem there was a variable degree

developed a non-fatal febrile illness when injected intracerebrally. Rabbits proved resistant. The virus passed through a Seitz filter. Judged by experiments with gradocol membranes its size was between 113 and 150 $m\mu$ (now given as 75 and 113 $m\mu$). It could be readily preserved by drying from the frozen state, but a saline suspension kept in the ice-chest proved innocuous within 13 days. It was destroyed by exposure to heat at 50° C. for 30 minutes. Antibodies specifically neutralizing the virus

phology of the individual cells. The movements of cells after fission and the arrangements that result determine, to a considerable extent, the texture of a colony and the shape of its edges. The long coiled filaments of the anthrax bacillus give the colony of this organism its characteristic "medusa-like" appearance. The presence of capsules on an organism gives its colonies a slimy consistency, and their absence a dry, roughened appearance. The colonies of some bacteria show coloring due to the elaboration of pigments by the organisms, but such pigments are not apparent in the individual cell under the microscope. Yellow and golden pigments are not uncommon, and others, including red, blue-green and violet, are met with from time to time.

ditions of life, and can be regarded as only one item in the sum total of characters that go to make up the concept of a bacterial species. One difference between colony types which has recently been discovered appears to be of deep-seated significance. In a number of bacterial groups it has been found that rough (R) and smooth (S) colonies will develop from a single, apparently pure, culture (p. 165). Under ordinary conditions, transfers from S and R colonies often breed true. The rough and smooth strains differ in virulence and other characteristics, and the marked and persistent association of colony characters with physiological and biochemical qualities may be of considerable biological significance.

BACTERIAL GROWTH

Although bacterial growth may be, and often is, assumed to be a simple process, both experimental evidence and theoretical considerations indicate that the problems of growth are relatively complex. It is convenient to consider bacterial growth under two general heads: first, the process of cell division or multiplication, and, second, the growth of bacterial populations.

Growth and Cell Division. By all odds the most common method of division is that of simple fission which divides the cell into approximately equal halves. Among bacilli and spirilla, cell division usually takes place at right angles to the long axis of the cell. The cocci may divide in one, two or three planes, thus giving rise to characteristic groupings such as chains, flat sheets or irregular masses, or cubical packets. Bacilli and spirilla show some elongation before division, cocci, as a rule, do not, although some exhibit an increase in the diameter of the cell without any alteration of its spherical form. The size which a single cell must reach before fission is, as among the higher forms of life, singularly constant for each species although, as will appear, some differences in this maximum size appear to be associated with the age of the culture.

Jour. Bact., 1949.) The center section is a light photograph of a young culture of *Bact*

critical illumination after Kohler. The chromatonic bodies are clearly apparent as the dark areas. (Robinson.)

following respects: (1) they produce a rapidly fatal disease in the mouse, hamster, and cotton-rat, a disease of variable severity in the guinea-pig, and an inapparent infection in the albino rat, rabbit, and rhesus monkey; (2) they are readily cultivated in the fertile hen's egg; (3) they are pantropic, rapidly appearing in the blood stream and reaching high concentrations in the viscera of susceptible animals; (4) under appropriate conditions viscerotropic strains cause acute interstitial myocarditis in the mouse characterized by necrosis of the muscle fibres and an inflammatory cellular reaction; (5) the lesions produced in the central nervous system are those of a diffuse poliomyelitis; (6) they are of small size, that of the Mengo virus estimated by filtration being 8-12 μ (Dick 1948), the Columbia S.K. virus estimated by electronmicrography at 25-30 μ (Jungeblut and Bourdillon 1943), and the encephalomyocarditis virus estimated by electronmicrography at 27-33 μ (Weil *et al.* 1952); (7) they agglutinate sheep red blood corpuscles (Olitsky and Yager 1949); (8) they appear to be natural parasites of the mouse, rarely infecting man; (9) they may be carried by mosquitoes.

Meningo-encephalitis of Unknown Origin.—It should be made clear that large numbers of sporadic cases of encephalitis occur in which the cause remains obscure. Some of these are undoubtedly manifestations of poliomyelitis, but the aetiology of others is unknown. Occasionally outbreaks are observed, such as those recorded by Jennings (1947) in England and Humbert and his colleagues (1947) in Tennessee. Further work is clearly required.

Secondary Encephalitis.—We have already (p. 2122) drawn attention to the encephalitis that occasionally follows vaccination against smallpox, and have discussed its aetiology. Similar cases of encephalitis sometimes occur during convalescence from other diseases such as measles, varicella, mumps, yellow fever, and influenza. The most striking characteristic of all these cases is the occurrence of perivascular demyelination—a lesion which is not usually met with in primary encephalitis. Whether the production of secondary encephalitis is due to the virus causing the primary disease, or to a different virus lying latent in the tissues, or is a purely degenerative condition not determined by a virus at all, has been a subject for debate (see McIntosh 1928, McIntosh and Scarff 1928, Greenfield 1930, Rivers 1932, Ledingham 1935, Hurst 1936). More recent work, which is reviewed by Hurst (1952), points strongly in favour of an allergic aetiology.

For a description of the clinical and pathological manifestations of the various types of encephalitis in human beings, the reader is referred to the monograph by Neal (1942).

ENCEPHALITIS IN ANIMALS

Borna Disease. Endemic Encephalomyelitis of Horses, Cattle and Sheep.

Borna disease is the name given to an infectious encephalomyelitis of horses. The name "Borna" is derived from the locality in Saxony where a severe epizootic occurred during the years 1894-96. The disease is widely distributed, and may occur in epidemic form with a mortality sometimes reaching 90 per cent. A similar encephalomyelitis is likewise observed in cattle and sheep. Histologically, lesions are found in the central nervous system and in the peripheral nerves, and characteristic inclusions (Joest-Degen intranuclear corpuscles) are observed in the large ganglion cells of Ammon's horn. The disease is due to a filtrable virus, which appears to be the same in all three species of animal. According to Elford and Galloway (1933b) it is about 85-125 μ in diameter. The virus is resistant to glycerol, but

While bacterial multiplication is commonly observed to take place by simple transverse fission, other modes of cell division may occur. Some forms, such as the diphtheria bacillus and the tubercle bacillus, show Y-like splitting and true branching characteristic of some of the higher fungi. Budding, similar to the budding of yeast cells, has been reported by some observers. More complex processes, such as the formation within a mother cell of a number of viable granules, called gonidia, which, upon liberation, develop into typical bacillary forms, have been reported by other workers. The status of the great majority of these observations has been open to some question since many of the structures reported were too small to have been resolved by the light microscope. The electron microscope, however, allows the resolution of

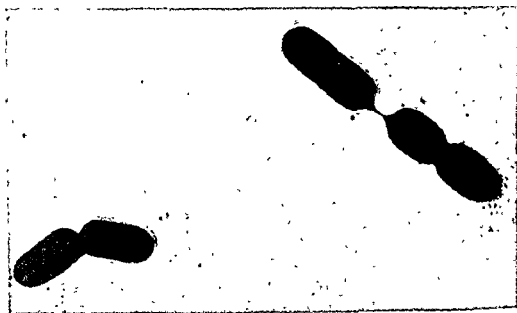


Fig. 15. Electron micrograph of *Lactobacillus acidophilus* showing various stages in cell division. The initial slight constriction, deeper constriction, and partially separated daughter cells are all shown. Note the light cell walls surrounding the dense black protoplast, and the diploid cells connected by a delicate strand of protoplasm. (Mudd, Polevitsky and Anderson, SAB No. 78.)

very minute structures and with it Smith, Mudd and Hillier²² have observed a kind of reproductive process in the *Bacteroides* (p. 540) and pleuropneumonia-like organisms (p. 548) in which these structurally fragile cells swell to form large round bodies that then may undergo multipolar germination. The outgrowing processes segment to form numbers of daughter cells. Whether such processes are of common occurrence among other kinds of bacteria is not clear.

The possibility of the occurrence of conjugation, and inferentially a sexual stage in reproduction, has been raised repeatedly but without adequate supporting evidence. Recently, however, Tatum and Lederberg²³ have reported the occurrence of mixed biochemical types of *Bact. coli* produced in mixed culture of strains derived from a single parent by x-ray irradiation and differ-

²² Smith, Mudd and Hillier *Jour. Bact.*, 1948, 56 603.

²³ Tatum and Lederberg *Jour. Bact.*, 1947, 53 673, Lederberg *Genetics*, 1947, 32 505.

of the cranial nerves is doubtful (see Hurst 1950). The horse is therefore *infective* for the mosquito during only a brief period. This is an additional reason for believing, as Tenbroeck, Hurst and Traub (1935) suggested, that the horse is not the primary reservoir of infection.

The cause of the western type of the disease was found by Meyer, Haring and Howitt (1931) to be a filtrable virus. A similar virus, differing from it immunologically was later shown by Ten Broeck and Merrill (1933), Howitt (1935), Records and Vawter (1935) and Shahan and Giltner (1935) to be responsible for the eastern type. Both viruses are estimated to be about 20-35 μ in diameter (Bauer *et al.* 1935, Tang *et al.* 1937), though Sharp and his colleagues (1943) give a figure of about 50 μ . They can be grown readily in tissue culture and on the chorio-allantoic membrane of the developing chick embryo. They remain viable in 50 per cent. glycerol in the ice-chest for a long time, but are readily destroyed by formalin. The eastern type is said to be more pathogenic than the western for guinea-pigs and rabbits, but both have a wide range of pathogenicity. Young animals are much more susceptible than old (Hurst 1950). The Argentine type of virus studied by Howitt (1935) appears to be identical with the western type, but the Venezuelan type, described by Beck and Wyckoff (1938) and Kubes and Ríos (1939), is immunologically distinct.

In protection against equine encephalomyelitis inoculation with a formalized chick embryo vaccine, using the technique of cultivation described by Higbie and Howitt (1935), appears to be hopeful (see Eichhorn and Wyckoff 1938, Fothergill 1941b).

Encephalitis of Rats.—A virus, possibly related to the eastern equine encephalitis virus, giving rise to encephalitis in rats, was recognized by Novy, Perkins, Chambers and De Kruif (1953) when studying *Treponema novyi* in 1909, and described by Jordan, Nungester and Preston (1953), who recovered it from blood that had been put into sealed tubes 40 years earlier. The virus is about 20 μ in diameter, and gives rise to the disease experimentally on intraperitoneal or intracerebral injection. After an incubation period of 3-6 days, there is anorexia, roughening of the coat, apathy, gradual inability to walk, glazing of the eyes, and slight hæmaturia. Death follows in 1-2 days preceded by convulsions.

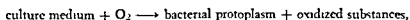
Encephalomyelitis of Mice.—This disease was described by Theiler (1937) in the United States, Gildemeister and Ahlfeld (1938) in Germany, and Iguchi (1939) in Japan. It is characterized by flaccid paralysis of the limbs and sometimes convulsions. Infection can be transmitted by intracerebral, intranasal and intraperitoneal inoculation. After intracerebral inoculation of mice there is an incubation period of 5-30 days. Flaccid paralysis then develops, usually affecting the fore limbs first, but being more severe in the hind limbs. Death usually follows 5-10 days after the onset of paralysis, but some animals survive and remain carriers for a time. Paralysed and carrier mice are resistant to a second intracerebral inoculation. There is evidence that immunity depends on the presence of neutralizing antibodies in the blood serum. Histologically, perivascular cellular infiltration is found throughout the central nervous system; there is acute necrosis of the ganglion cells, particularly in the anterior horn, followed by neuronophagia. After intramuscular inoculation there is myositis. No inclusion bodies appear to have been demonstrated yet.

The virus is one of the smallest known—9 to 13 μ —resembling in this respect the virus of poliomyelitis. It is readily destroyed at temperatures above 50°C,

ing in growth requirements. It is very difficult to interpret this evidence on other than the basis of a gene recombination arising through conjugation between the cell types. These observations have been confirmed by Haas, Wyss and Stone,²⁴ who have also observed an apparent fusion of chromatinic bodies on recovery from irradiation. Whether this last is to be interpreted as cytological evidence of a nuclear fusion and recombination of mutant characters is not clear.

While, then, a number of kinds of reproduction occur among the bacteria, binary fission is by far the most common. Under favorable conditions cell division may take place quite rapidly, as short a time as twenty minutes elapsing between one division and another of the colon bacillus. This rapidity of cell division is sometimes referred to as if it were a peculiar quality of bacteria, but as a matter of fact the embryonic cells of many higher forms of life divide quite as rapidly as bacteria. The remarkable thing about bacterial cell division is not so much the rapidity with which one cell division succeeds another as the fact that a very short time suffices for the growth of the young cell to maturity. A young bacterial cell attains full size and acquires the capacity to produce, in its turn, an independent organism much sooner than most other forms of life. This rapid reproduction of distinct individuals is plainly different from the multiplication of embryonic cells among higher organisms.

The Growth of Bacterial Populations. For obvious practical reasons bacteria are ordinarily manipulated and studied in groups consisting of large numbers of individuals. Likewise, bacterial growth is of practical significance, not in terms of one, two or three cell divisions, but as growth in populations which often reach a density of three to four hundred million cells per cubic centimeter of culture fluid. Growth of these organisms in culture may be considered from a variety of points of view, the particular one adopted being dependent upon the nature of a given investigation. A simple view that is commonly, if tacitly, taken assumes that growth may be described by the reaction



which proceeds from left to right and eventually comes to an equilibrium. The reaction velocity constants may be determined either by the rate of disappearance of the reacting substances or the rate of appearance of end products. Although complicated by a variety of side reactions, the assumption of such a relation has been extremely useful in the studies on bacterial metabolism. The growth of autotrophic bacteria, for example, is conveniently measured in terms of the conversion of carbonate to the organic carbon of protoplasm. A somewhat more mechanistic concept is that of the instability of an inoculated culture medium which, after undergoing a series of complex changes, reaches a stable equilibrium at a considerably lower energy level. Such a concept is, of course, the basis of studies of the energy metabolism of bacteria. These and similar views are oversimplified and, although of undoubted value, present only limited aspects of the phenomena of bacterial growth.

²⁴ *Proc. Nat. Acad. Sci.* 1948, 34 229.

The virus is about 10-15 μ in diameter. It seems to be moderately resistant to drying, and is destroyed by heating at 70° C. for 30 minutes (Fortner 1942). During life it can be demonstrated in the cerebrospinal fluid of sick animals, and occasionally in the faeces during the pre-paralytic stage, by inoculation into pigs. It has so far not been cultivated *in vitro* or in the fertile egg. In animals infected by the mouth it is said to be present in the blood during the early part of the incubation period, though not later. Animals that recover have neutralizing antibodies in the serum and are permanently immune. Their serum, however, has apparently no protective or curative action as ordinarily administered. The value of protective vaccines is doubtful. The slaughter policy is recommended by Klobouk (1952). (For further information see Fortner 1942, and Kaplan and Meranze 1948, who review the disease.)

Encephalomyelitis of Coypus.—Wenkebach (1952) described the epizootic occurrence of acute and chronic encephalomyelitis in the coypu (*Myocastor coypus*)—the nutria of the fur trade—in Argentina. It is caused by a virus named the *Maruen virus* after the neighbourhood in which it was first recognized. In man the virus produces a disease often diagnosed as influenza. It is pathogenic for mice, guinea-pigs, rabbits and cats. It can be grown in the fertile hen's egg. In sick animals it is found in the central nervous system, blood, lungs, liver and spleen. Histologically intranuclear and intracytoplasmic acidophilic Feulgen-negative inclusion bodies are demonstrable in the brain.

Infectious Paralysis of Guinea Pigs.—This disease occurs sporadically in guinea-pigs, and closely resembles poliomyelitis. According to Romer (1911), who first described it, it can be transmitted to normal guinea-pigs by the intracerebral inoculation of filtered or unfiltered brain or cord suspensions. After an incubation period of 9 to 22 days, the animals become febrile and lose weight; nervous disturbances develop, consisting of a gradually increasing muscular weakness, particularly of the hinder parts of the body; paralysis of the hind legs develops, and occasionally of the bladder. The disease lasts from 3 days to 3 weeks. Pathologically, the lesions are those of a disseminated meningo-myeloencephalitis. They differ from those of poliomyelitis in showing greater meningeal infiltration, and in being most severe around the central canal of the cord. The virus is constantly present in the central nervous system; it is also found in the prevertebral, inguinal, and mesenteric lymph glands, and occasionally in the liver and spleen.

Infectious Encephalomyelitis of Chickens.—This disease, which is sometimes referred to under the name of "epidemic tremor of chickens," was reported by Jones (1934) in New England. It is characterized by tremor, most noticeable in the head and neck, and progressive ataxia, either or both of which may be present in a single bird. Young chickens are mainly attacked, the usual age of onset being about 3 weeks. In commercial flocks the morbidity ranges from 5 to 50 per cent. In severe outbreaks about half the chicks may die; death is due to inanition caused by the progressive ataxia. Histologically, focal collections of glia cells, perivascular infiltration, and degeneration of nerve cells, especially of the Purkinje cells in the cerebellum, are found in the central nervous system. No inclusion bodies have yet been demonstrated. The disease can be reproduced in young chicks by intracerebral inoculation of brain and cord from affected birds. The incubation period of the experimental disease is usually 2-4 weeks; death follows about a week after the onset of symptoms (van Roekel *et al.* 1938). The infective agent is probably fairly large, since it can pass through a Berkefeld N

The existence of bacteria in large aggregations or *populations* is of no little significance because *ipso facto* they are subject to the principles of population mechanics. The potential multiplication of these organisms by geometrical progression may be realized only up to a certain point. As numbers increase in the microcosm of the test tube, competition between individual organisms for foodstuffs, oxygen and the like progressively reduces the opportunity for further growth until a saturating population is reached.

If no increasingly effective retardation were operative, the potential increase in a population would be expressed by the relation

$$\frac{dY}{dt} = bY$$

where Y is equal to the number of individuals per unit volume and b to the potential rate of multiplication of each organism. When, however, there is a maximal possible population, K , this potential geometric increase is only partially realized, the extent of the realization depending on how near the size of the population is to its maximum at any given moment. Or, mathematically,²⁵

$$\frac{dY}{dt} = bY \frac{K - Y}{K}$$

This equation is the differential equation of the logistic curve the mathematical form of which is.

$$Y = \frac{K}{1 + e^{a - bx}}$$

This function, plotting as a symmetrical sigmoid curve, has been found to describe, with a high degree of precision, the growth of populations of a variety of organisms including man, yeasts, *Drosophila*, certain protozoa, etc.

If the numbers of bacteria in a growing culture are determined from time to time and plotted against time, the points fall on a similar sigmoid curve. The resulting curve is, however, asymmetrical in that the point of inflection is not halfway between the upper and lower asymptotes but is considerably near the lower asymptote. This discrepancy between bacterial populations and populations of other organisms has not as yet been satisfactorily explained.

Partly as a consequence of this discrepancy and partly as a result of the large error inherent in the enumeration of bacteria, the treatment of bacterial growth curves has taken a slightly different path. Such curves are ordinarily not plotted arithmetically but on semilog paper; *i.e.*, the logarithms of the numbers of bacteria are plotted against time. This procedure has the advantage that not only are the errors of enumeration minimized but, when the organisms are multiplying at a geometric rate, the points fall on a straight line and the generation time determines the slope of this line. Furthermore, the numbers of viable bacteria, as determined by plate count, decline not long after the maximum population has been reached and the organisms appear to die off at a geometric rate. The equilibrium predicted by the logistic function is

²⁵ For a more detailed discussion of such rationalization the student is referred to Lotka's *Principles of Physical Biology*. Williams & Wilkins Company, Baltimore. 1925.

(see p. 2035 and Pool *et al.* 1930, Alston and Gibson 1931, Brownlee and Wilson 1932, Gordon *et al.* 1932, MacLeod and Gordon 1932, Gordon 1934). The disease may also be transmitted to monkeys (Hurst 1931a) and to field voles (Findlay and Elton 1933). Both mice and monkeys can be infected by intranasal as well as by intracerebral inoculation (Elford and Galloway 1933a, Galloway and Perdrau 1935). The virus is about 15–20 μ in diameter (Elford and Galloway 1933a). It can be cultivated in the fertile egg and in chick embryo tissue culture. It may infect man and give rise to meningo-encephalitis, usually after a prodromal illness resembling influenza (see Lawson *et al.* 1919). Most of the cases have occurred in laboratory workers. Neutralizing antibodies are detectable in the patient's serum within a few days and persist for years. Diagnosis may be made during life by injection of the blood or cerebrospinal fluid into mice. Sheep may be actively immunized by means of a 0.35 per cent formolized vaccine made from sheep brain, cord, and spleen, but there is a danger of conveying scrapie (see p. 2187) by such a vaccine (Gordon 1916). Passive immunity may be conferred on lambs born of non-immune mothers by subcutaneous injection of antiserum at birth (Wilson and Gordon 1948). For human beings exposed to special risk of infection a vaccine prepared from formolized mouse brain is suggested (Edward 1918).

LYMPHOCYTIC CHORIOMENINGITIS PSEUDO-LYMPHOCYTIC CHORIOMENINGITIS DURAND'S DISEASE

These three diseases may be considered together. In 1925 Wallgren coined the term "acute aseptic meningitis" to comprise cases of an acute febrile disease, accompanied by meningeal irritation and sometimes by inflammation of the upper respiratory tract, terminating favourably within 10–14 days. Since then benign lymphocytic meningitis, as it is sometimes called, has been found to be of varied aetiology. It is sometimes met with, for example, during the course of mumps and glandular fever, it is not at all an uncommon manifestation of human infection with *Leptospira canicola*; and it is a dominant feature in the disease of swineherds (see p. 2054), which is caused by *Leptospira pomona*. In this section we shall deal with the virus of (1) lymphocytic choriomeningitis, which has been reported chiefly from the United States and occasionally from other countries; (2) pseudo-lymphocytic choriomeningitis, which has been isolated on only two occasions—both in Great Britain, and (3) Durand's disease, of which only one case has been recognized—in Tunis.

Lymphocytic Choriomeningitis.

Armstrong and Lillie (1934), while investigating a fatal case of St. Louis encephalitis, isolated a virus, quite different from the ordinary St. Louis virus, which gave rise in experimentally inoculated monkeys and mice to a lymphocytic choriomeningitis. An apparently identical virus was isolated by Scott and Rivers (1936) (see also Rivers and Scott 1936) from two benign cases of non-bacterial lymphocytic meningitis in adult males in the United States, and by Findlay, Alcock, and Stern (1936) in this country from the cerebrospinal fluid of two patients who exhibited vague nervous symptoms following a febrile attack. A similar or identical virus was isolated by Findlay and his colleagues from an irradiated mouse showing

not realized unless experimental conditions are such that the food supply is constantly renewed and the waste products are removed. Such an equilibrium is attained, for example, in cultures in constantly flowing culture medium and the viable count tends to attain and persist at a constant level though the total count continues to rise, i.e., multiplication balances the death rate, but the dead cells accumulate.²⁶ A graphic representation of the rise and decline in numbers of viable bacteria in culture is given in Fig. 16.

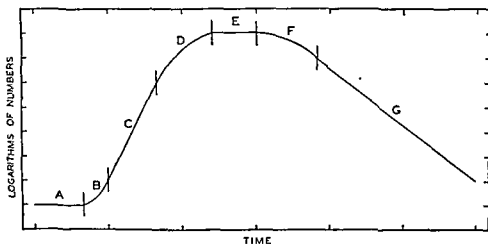


Fig. 16 Diagrammatic representation of a bacterial growth curve, numbers of viable organisms plotted against time of incubation. A, initial stationary phase, B, positive growth acceleration phase, C, logarithmic growth phase, D, negative growth acceleration phase, E, maximum stationary phase, F, phase of accelerated death, G, logarithmic death phase. (After Buchanan.)

Buchanan²⁷ has divided this curve up into seven parts which he designates as follows.

- A *Initial Stationary Phase*. During this phase the number of bacteria remains constant.
- B *Lag Phase or Positive Growth Acceleration Phase*. During this phase the generation time decreases progressively until a minimum is reached.
- C *Logarithmic Growth Phase*. In this phase the generation time remains constant and the organisms increase in numbers by geometrical progression.
- D *Negative Growth Acceleration Phase*. The generation time progressively increases during this phase and the bacteria continue to multiply but at a decreasing rate.
- E. *Maximum Stationary Phase*. Here the numbers of viable bacteria are at a maximum, neither increasing nor decreasing.
- F *Phase of Accelerated Death*. Following the stationary phase, the numbers of viable bacteria decrease, slowly at first but with increasing rapidity, until a relatively constant rate is attained.
- G *Logarithmic Death Phase*. During this phase a constant rate of death is maintained. It should be noted that this rate is not maintained indefinitely for the curve tends to become asymptotic to the X axis.

Different explanations have been offered to account for the varying growth rates observed in bacterial cultures. The failure of inoculated organisms to

²⁶ Jordan and Jacobs. *Jour. Bact.*, 1944, 48:579.

²⁷ Buchanan. *Jour. Inf. Dis.*, 1918, 23:109. See the review by Hinshelwood. *Biol. Rev.*, 1944, 19:150.

antigen of protein nature can be demonstrated in the virus by precipitin and complement-fixation reactions (Smadel *et al.* 1939, 1940). It appears to be different from the antigen that reacts with neutralizing antibodies in immune serum.

Diagnosis.—The cerebrospinal fluid is usually under pressure and contains an excess of lymphocytes, though this is not invariable. The globulin is often increased; the sugar may be low and the chlorides normal. The virus can be demonstrated in the blood and throat washings, or in the cerebrospinal fluid soon after the onset of meningeal symptoms, by inoculation intracerebrally into mice derived from a known non-infected stock, and its identity may be determined by neutralization with specific sera or by a specific complement-fixation reaction (Smadel and Wall 1941). Indirectly, the diagnosis may be assisted by the demonstration of complement-fixing antibodies (see Casals and Palacios 1941) or of neutralizing antibodies (Armstrong and Wooley 1935, Wooley *et al.* 1937) in the patient's serum. Complement-fixing antibodies appear within the first 3 weeks, neutralizing antibodies not for 6-10 weeks. Serum should be taken at the beginning of the disease, and again after 4-12 weeks according to the test that is to be used. Unlike the complement-fixing antibodies, which disappear within a few months, neutralizing antibodies persist for years.

Pseudo-lymphocytic Choriomeningitis.—Only two cases of this disease have so far been described. Clinically, they resembled benign lymphocytic choriomeningitis. MacCallum and his colleagues (1939) isolated a virus from the cerebrospinal fluid of each of the patients. It was considerably larger in size—150 to 225 μ —than the true choriomeningitis virus. It gave rise in mice, guinea-pigs, and *rhesus* monkeys to a clinical and pathological picture very similar to that of choriomeningitis, except that in mice inoculated intracerebrally the incubation period was shorter—4 to 5 days instead of 5 to 12 days—and the pathological lesions were less extensive. Judged by cross-immunity tests in mice, the two viruses appeared to be distinct.

Durand's Disease.—Durand (1940) described a febrile disease in himself, which he showed to be caused by a virus. Findlay (1942), who likewise contracted the disease while working with the virus in the laboratory, suggested the name Durand's disease. The symptomatology appears to be variable. Fever lasts about 10 days, and is accompanied by headache, and sometimes by symptoms of meningeal, upper respiratory or gastric inflammation. Blood taken during the fever is infective for guinea-pigs, occasionally the urine or cerebrospinal fluid may also be shown to contain the virus. Guinea-pigs are susceptible to inoculation by practically any route. After subcutaneous injection there is an incubation period of about 6-10 days, which after one or two passages of the virus falls to 2-5 days. There is fever lasting 6-10 days, local enlargement of the lymph nodes, dyspnoea, severe loss of weight, and a leucopenia after a preliminary leucocytosis. Only about 10 per cent. of the animals die—usually after 3-4 weeks. Animals killed a week or so after the onset of the fever show emaciation, a local hæmorrhagic gelatinous œdema, great enlargement of the spleen, some degree of interstitial pneumonia, and sometimes a brownish-yellow discoloration of the liver. The virus is present in the blood and organs. Intratesticular inoculation of guinea-pigs produces an orchitis. Animals that recover from the disease are immune. Cats, dogs, and *rhesus* monkeys show some meningeal reaction after intracerebral inoculation, but most other animals develop only a latent infection. Two human patients inoculated subcutaneously by Durand for purposes of pyrotherapy became febrile in 4 days;

begin multiplication at the maximum rate at once has been attributed both to environmental factors and to the physiological state of the organisms. For example, it has been assumed that a partial pressure of carbon dioxide is essential to cell division, for, when it is prevented from accumulating by gassing with CO₂-free air or nitrogen, growth does not take place. On the other hand, the stationary and lag phases are said to be entirely eliminated by inoculation from a culture which is in the logarithmic phase of growth. Hinshelwood²⁸ has presented convincing evidence of a true and apparent lag phase, the latter resulting from the admixture in the inoculum of dead and dying cells with those capable of active proliferation. He suggests that the lag may be attributed to the time necessary for the accumulation of enzymes, diffusible coenzymes and essential intermediate compounds in the synthesis of cell substance (p. 185) to concentrations at which synthesis may occur at a maximum rate. The lag phase is of particular interest in connection with the action of drugs on bacteria (p. 152), since the bacteriostatic drugs produce an effect which is seemingly that of an indefinitely prolonged lag phase.

Hershey²⁹ has developed mathematical equations by which the latent period in multiplication and the numbers of organisms expected at any time during the phase of rapid growth may be predicted. Since only differences in the size of the cells are postulated (see below), the satisfactory agreement of experimental data with calculated values has undoubtedly more than accidental significance.

During the logarithmic growth phase the number of cells increases at a constant and practically exponential rate, for the mortality, under favorable conditions, is very low, more than 90 per cent of the cells continuing to divide. The rate of multiplication is independent of food concentration and of the effects of toxic substances over a relatively wide range, though at critical concentrations the activity of the latter increases sharply and contributes in large part to deceleration at the end of the logarithmic phase. The logarithmic growth phase, then, may be regarded as a steady state in which enzyme and substrate concentrations are maintained at such a level that the rate of synthesis of cell substance proceeds at the maximum for the strain of bacteria in that environment.

Likewise, the decreasing growth rate in the negative growth acceleration phase has been accounted for in various ways, including the rate of diffusion of oxygen into the medium, the accumulation of toxic by-products such as organic acids and ethyl alcohol, etc. It is probable that different factors are operative with different microorganisms and with the same microorganism under different conditions, and that each culture constitutes a special case.³⁰

The mechanisms operative in bacterial death, both in culture and in the presence of antiseptics, are uncertain. The survivor curves are logarithmic, i.e., the same percentage of viable organisms is dying at any particular moment. The fact that this rate may be described by a mathematical expression which likewise describes a monomolecular chemical reaction is generally held to be

²⁸ Hinshelwood *Chemical Kinetics of the Bacterial Cell*. Clarendon Press, Oxford. 1946.

²⁹ Hershey. *Jour. Gen. Physiol.* 1939, 23.11.

³⁰ Henrici *Morphologic Variation and the Rate of Growth of Bacteria*. Charles C Thomas, Springfield and Baltimore. 1928.

experiments of Lurie (1942), who injected tubercle bacilli into normal and immune rabbits, and two days later removed specimens of tissue, from which he obtained suspensions of mononuclear phagocytes containing ingested tubercle bacilli. These were injected into the anterior chamber of the eye of normal rabbits, "immune" cells into one eye, "normal" cells into the other. After 10-14 days "normal" cells had proliferated, and the chamber contained large numbers of intracellular and extracellular tubercle bacilli; "immune" cells had not proliferated and only a few degenerate bacilli were seen, mostly intracellular. Viable counts of the contents of the anterior chamber confirmed the inference that the "immune" cells inhibited the growth of tubercle bacilli. It is not, however, clear how far the increased immunity was specific for tubercle bacilli. For example, in earlier studies Lurie (1939) showed that macrophages from tubercle-immune animals were more actively phagocytic *in vitro* than those from normal animals, but that the activity was displayed, not only towards tubercle bacilli, but towards inert particles and other bacteria. Suter (1953) recorded a similar enhancement of the ability of tissue-cultures of guinea-pig and rabbit monocytes to suppress the intracellular multiplication of tubercle bacilli, in animals immunized with B.C.G.

Perhaps the strongest argument in favour of the essential rôle of the serum antibodies in antibacterial immunity is the fact that such immunity is specific. It is true that a non-specific active immunity can sometimes be induced; but it is almost always moderate and transient (see Chapter 52). Effective and durable active immunity is usually narrowly specific; and in many cases we can identify the particular antigenic component, or components, on which it depends. There may be a specific immunity whose induction, like that of antibody immunity, is associated with the presence of a characteristic component of the bacterium, and which has nothing to do with antibody formation; we must then invoke an additional specific mechanism, in which the same antigenic components are in some way involved. There is nothing very unlikely in such an assumption, and it may well prove to be correct, but it will accord with the sound principle of economy of hypothesis, to reserve judgment pending the accumulation of further evidence.

The Dependence of Effective Antibacterial Immunity upon Particular Antibodies.

On which of the various serum antibodies does antibacterial immunity depend? This question was answered, in respect of one particular infection, long before anyone realized that it had been asked. Neufeld and Händel (1909), in their classical paper on the testing of antipneumococcal sera, first showed the existence of different serological types of pneumococci by noting that a given serum would protect a mouse against one strain of pneumococcus but not against another.

The demonstration of the presence of two separable antigenic components in the pneumococcal cell—the type-specific polysaccharide and the non-specific nucleoprotein—afforded an obvious opportunity of approaching this problem along more satisfying lines; and the results obtained were unequivocal. The antibody corresponding to the polysaccharide hapten was found to have a high protective value: the antibody corresponding to the nucleoprotein antigen had little, if any, protective effect (Avery and Morgan 1925, Avery and Neill 1925). A decisive demonstration of the protective action of the antibody acting on the specific polysaccharide, in the absence of antibodies acting on any other constituent or product of the pneumococcal cell, was made by Avery and Goebel (1931). They prepared a synthetic antigen by linking a purified polysaccharide from a culture of Type III pneumococcus to horse globulin, and with this antigen they immunized rabbits. The antisera so obtained protected mice against Type III pneumococci, but not against Type I or Type II pneumococci.

nothing more than a fortuitous coincidence, and the conclusion drawn by some workers that bacterial death is a monomolecular reaction has no basis in fact.

Morphologic Variation During Growth.³⁰ It has been observed that both the morphology and the physiological activity of bacteria differ in different phases of the growth of a culture. As growth begins to get under way, the maximum size that a given cell reaches before fission increases somewhat, and microscopic examination shows that a large proportion of the bacteria in a culture at this stage of development are appreciably larger than at other times. The cells stain evenly, and there is no evidence of granular structure even in those organisms in which metachromatic granules are most readily demonstrated. The rate of respiration per cell increases, reaching a maximum at the end of the phase of accelerating growth and declining as the culture goes into the logarithmic growth phase. The increased metabolic rate in the early growth stages is, however, more apparent than real and has been shown³¹ to be quantitatively related to cell size. The cell size declines also as the culture goes into the logarithmic growth phase, although the individual cells remain homogeneous and take stains evenly. By the time the stationary phase is reached, the bacterial cells are uniformly smaller and in cultures of spore-forming organisms, many are forming spores. As the viable bacterial count decreases the cells no longer stain uniformly but show a granular structure and involutionary forms appear. This succession of morphologic types, the embryonic or young cells, the mature forms and finally the senescent forms, has been designated *cytomorphosis*.³⁰ It is possibly analogous to a similar succession of events in the higher forms of life.

THE CHEMICAL COMPOSITION OF BACTERIA

The chemical composition of bacteria is not greatly different from that of other living material. They differ in composition from species to species, and the chemical constitution of a given species is influenced to a considerable extent by the composition of the medium upon which the organisms are grown. In consequence, a precise statement of the relative amounts of the compounds and elements of which these organisms are composed is not possible, only crude approximations may be made.

Bacterial cells contain considerable quantities of water but somewhat less than the cells of the higher plants and animals. Estimates of water content vary widely, very likely owing to the difficulty of getting accurate wet weights. Although some organisms have been reported to contain as much as 90 per cent water, most bacteria show, in the hands of careful workers, 70 to 80 per cent water. In some species of bacteria an appreciable part, 17 to 28 per cent, of this is bound water.³² The ash content, i.e., the inorganic material remaining after combustion of the cells, depends to some degree, both qualitatively and quantitatively, on the inorganic content of the medium on which the organisms are grown. The values are, therefore, subject to considerable variation, and have been given as from 2 to 14 per cent of the dry weight of the cells. The ash is largely phosphoric acid, the P_2O_5 content ranging from 10 to 45 per cent of the total ash. Undoubtedly a considerable proportion of this

³¹ Hershey and Bronfenbrenner. *Jour. Gen. Physiol.*, 1938, 21:721.

³² Cf. Friedman and Henry: *Jour. Bact.*, 1938, 36:99.

outbreak in 1917, when the attack rate was 18 per 100,000. In the epidemic of 1921 in Iceland an attack rate of 470 per 100,000 was registered. The disease resembles cerebrospinal meningitis in affecting only a small proportion of the population; though, as in meningitis, there is reason to believe that the disease is often widespread in a sub-clinical or latent form. Several workers on the basis of epidemiological or virological evidence have concluded that under endemic conditions there are something like 100 latent cases to every paralytic case (Stocks 1932, Howe 1949, Brown *et al.* 1949). In Chicago it was estimated that most children had probably been infected once by their 4th birthday and 75 per cent. twice by their 6th birthday, even though only a small proportion of them experienced the paralytic form of the disease (Casey *et al.* 1950); and that in Baltimore in 1917-8 there were probably 1,000 latent infections to every clinical case (Turner *et al.* 1950). The case-fatality rate of the declared disease varies considerably, but is usually about 10-15 per cent. It is lowest in infancy and rises with increasing age. Thus for Sweden between 1925 and 1944 Olm and Heinertz (1951) give the figures quoted in Table 178. The proportion of cases that show residual paralysis is difficult to estimate, because there is an increasing tendency among clinicians to diagnose the disease in the pre-paralytic stage. In this way a considerable number of abortive cases are included that do not develop paralysis at all. Probably not more than about 15-40 per cent. of the total cases show residual paralysis, and in many of these partial or even complete restoration of function ultimately occurs. Males are affected more often than females.

TABLE 178

CASE-FATALITY RATE OF POLIOMYELITIS IN SWEDEN 1925-44 ACCORDING TO AGE.

Age Group in Years	Paralytic cases	Case-fatality Rate %
0-	1,145	3.8
3-	2,221	5.5
7-	4,097	10.3
15-	4,551	17.2
Over 25	3,596	23.7
All ages	15,610	14.2

In the past poliomyelitis has been largely a disease of infancy and early childhood, but of late years the older age groups have been progressively more and more affected (see Burnet 1940). Thus, in the Danish outbreak of 1934 no fewer than 33.2 per cent. of the cases were in persons aged 15 years or more (Jensen 1935). There is reason to believe that the attack rate in persons infected for the first time is lowest in infancy and highest in adolescence and young adult life. In countries with a low level of sanitation the disease, mainly in the non-paralytic form, is prevalent in infants and young children. On the other hand, in countries with a high level of sanitation, little sub-clinical immunization occurs in early life, so that the average age of infection tends to be later and, as the incidence of paralysis rises with age, the paralytic form is seen more frequently (see Sabin 1947b, Nelson 1947, Burnet 1952). For example in the outbreak in Malta in 1912-3 cases were restricted mainly to children of the indigenous population and to members of the British armed services; the adult Maltese escaped almost completely (Seddon *et al.* 1945). For the same reason the incidence of the disease tends to be higher

phosphorus exists in the cell in the form of nucleic acids. Sulfur, potassium, chlorine and calcium are also present in notable amounts, together with usually smaller quantities of magnesium, iron, silicon, etc.

There is a good deal better agreement regarding the carbon content of bacteria, for most analyses give about 50 per cent of the dry weight as carbon. A part of this, of course, is present in the cell as protein, but the polysaccharide gums which make up the capsular material account for a considerable portion. Cellulose is not a common constituent of bacteria but has been identified³³ Hemicellulose is often present, and starchlike material (granulose) is found within the cells of some species.

The total nitrogen of bacterial cells varies more widely. An estimate of 8 to 15 per cent of the dry weight is in accord with the majority of analyses. A part of the nitrogen is present in the cell as protein, but very little is known of bacterial proteins. Both albumins and globulins have been isolated, the latter much more frequently. A fraction obtained by extraction by dilute alkali and called nucleoprotein has been of immunological interest and has been studied more thoroughly than other protein constituents. It appears to be a globulin combined with nucleic acid. In amino acid content the bacterial proteins do not differ materially from plant and animal proteins, and, in contrast with inorganic salt content, the amino acid composition of a given bacterium is a highly stable character and unaffected by variation in the culture medium.³⁴

A finding in keeping with the high phosphorus content of bacterial ash is the relatively large proportion of the total nitrogen that may be accounted for as nucleic acid. These organisms contain a higher proportion of nucleic acid than any other tissue except thymus, a characteristic they share with the yeasts and molds. As much as 7.1 to 11.5 per cent of the total nitrogen is purine nitrogen. Adenine, guanine, cytosine and thymine have been isolated from the tubercle bacillus, the presence of thymine and the absence of uracil suggesting the animal type of nucleic acid. Guanine has been isolated from the colon bacillus and cytosine and uracil from the cholera vibrio. Thymine is absent from the latter, suggesting in turn the plant type of nucleic acid. In addition to the nucleic acids proper (polynucleotides), their decomposition products occur in the cell, including mononucleotides, nucleosides and the free bases. The structure of bacterial nucleotides is uncertain although the predominating base is adenine. The function of adenylyl pyrophosphate as a phosphate carrier in fermentation is discussed elsewhere (p. 90).

Some bacteria contain considerable quantities of fats, lipids and waxes, *i.e.*, ether extractable material. The variation among bacteria as a group is great, analyses indicating that from 2 to 40 per cent of the dry weight may be lipid in nature. Here, however, the variation within a species is not so great, but certain species, such as the acid-fast bacteria, contain large amounts of ether extractable material. These, for the most part waxes and complex alcohols, appear to be associated with the acid-fast staining properties of these organisms.

³³ Hibbert and Barsha: *Jour. Amer. Chem. Soc.*, 1931, 53:3907.

³⁴ Stokes and Gunness: *Jour. Bact.*, 1946, 52:195; Freeland and Gale. *Biochem. Jour.*, 1947, 41 135.

be found in the faeces, not only of patients, but of contacts during an outbreak. Thus, Langmuir (1942) found it in the faeces of 4 out of 5 cases and of 20 out of 27 intimate contacts. The studies of Pearson and his colleagues (Pearson and Rendtorff 1945, Pearson *et al.* 1945) seem to show that the virus is restricted very largely to cases and close contacts. Thus, during an epidemic at Forth Worth, Texas, in 1943 the virus was demonstrated in the stools in 6 out of 8 households containing 27 familial contacts, in 8 out of 45 households containing 80 non-familial contacts, and in only 2 out of 127 households containing 374 non-contacts. Similarly, Kessel and Moore (1945), who examined tonsils and stools from non-contacts during an inter-epidemic period, isolated the virus from only 5 out of 136 persons studied (see also Francis and Brown 1948, Casey *et al.* 1950). Sabin and Ward (1941) were unable to find the virus in saliva. At post-mortem it can be demonstrated in the spinal cord, in parts of the intestinal tract, and sometimes in the lymph nodes (see Wenner and Paul 1947), but not in the cerebrospinal fluid or urine. For a long time it was believed to be absent from the blood, but there is increasing evidence that in man, in chimpanzees, and in *cynomolgus* monkeys it may be present during the pre-paralytic stage of the disease (see Koprowski *et al.* 1947, Horstmann 1952a, Bodian 1952a, Bodian and Passenbarger 1954).

The interpretation of these findings is difficult. They must be related to the epidemiological picture. In spite of the fact that the virus is present in the intestinal tract, is excreted in the faeces, is often present in sewage (Paul *et al.* 1940, Paul and Trask 1941, 1942, Melnick 1947, Rhodes *et al.* 1950b), and has been isolated from flies (Trask *et al.* 1943, Ward *et al.* 1945; see also Melnick and Penner 1952), the disease does not behave like a water-borne disease (see Maxcy 1943). It is true that a few milk-borne outbreaks are on record (see Aycock 1941, Goldstein *et al.* 1946), but it is well known that diseases of the respiratory tract, such as scarlet fever and diphtheria, may occasionally be carried by milk. In most outbreaks that have been carefully studied, a history of close contact with cases or with persons closely associated with cases has been established (see Burnet 1940, Aycock and Kessel 1943, Casey 1945, McFarlan *et al.* 1946, Silverthorne *et al.* 1949, Tyrrell 1951). The closeness of the association necessary is illustrated by the outbreak at Eccles, where the disease was confined for 6 weeks to one-half of the borough by a narrow canal (Sweetnam 1948). This epidemiological evidence is in general conformity with the laboratory evidence which shows, as noted above, that the virus is largely restricted to cases and close contacts of cases. It seems probable, therefore, that infection is transmitted mainly by intimate contact with the pharyngeal secretions of patients near the beginning of the illness or with the hands of patients or carriers soiled with pharyngeal or faecal material (see Dauer 1947).

The statements that infection occurs mainly by intimate association with cases, and that the virus can seldom be found except in cases and close contacts, may seem at variance with the previous statement that under endemic conditions there are estimated to be 100 latent infections to every clinical case. The discrepancy is more apparent than real. For example, at Forth Worth, Texas, 10 cases of poliomyelitis occurred in a population of 200,000. If 100 persons were infected to every clinical case, then there must have been 1,000 latent infections. Pearson and his colleagues (1945), who examined a sample of 374 persons without a history of contact, found 2 faecal carriers—a number agreeing closely with the expected finding of 1·8. It must also be remembered that the history of intimate association refers particularly to paralytic cases. It may well be that less intimate contact may give rise to infection of a degree insufficient to cause the fully developed clinical disease.

How the virus reaches the central nervous system after gaining access to the

BACTERIAL PHYSIOLOGY^{1 2}

The biochemical changes brought about through the activities of bacteria are by far the most obvious manifestations of their existence. The long-known but little understood phenomena of fermentation, putrefaction and decay, soil fertility and infectious diseases of man and lower animals are, in essence, no more than the exhibition of one or more of the many facets of the biochemical potentiality of these microorganisms. The oxidation of ammonia by the nitrifying bacteria, the decomposition of carbohydrates to alcohols and acids and the production of substances toxic to higher animals are a part of the normal activities of this heterogeneous group of living organisms. The elucidation of many of these phenomena has not only made possible a partial control of a not inconsiderable portion of man's environment but has provided an insight into the life processes of these organisms, organisms structurally so simple that morphology cannot carry us far in their study.

The term bacterial physiology, or, sometimes, bacterial metabolism, is a broad one which is generally assumed to include the entire sequence of events taking place in a bacterial culture. Since the small size of the individual bacterial cell precludes the utilization of many of the physiological techniques adapted to the multicellular, differentiated organism, the physiology of bacteria becomes, for all practical purposes, a biochemical physiology. The very magnitude of the biochemical changes brought about by these organisms tends to reinforce a belief in their relative importance, bacteria have, for example, been shown to consume forty to sixty times as much energy as man in terms of calories per gram of body nitrogen. The obvious significance of physiological activities is so readily apparent that it has tended to obscure to many the undoubted importance of the environmental factors that influence the functioning of the living cell. It is to be borne in mind, therefore, that although bacterial physiology is a branch of general cellular physiology, it must be regarded as a special case which, in many instances, goes considerably further afield into other fundamental aspects of biology than the present concept of cellular physiology is generally assumed to do.

¹ For general and more detailed discussions see: Buchanan and Fulmer: *Physiology and Biochemistry of Bacteria*, Vol. I, II and III, 1933, McGraw-Hill, New York.

Moulder, who also revised the section on autotrophic metabolism

and the animal easily becomes tired. Within a few hours ataxia develops, accompanied by tremor of the arms, legs or head. The ataxia rapidly merges into a flaccid paralysis, which extends to practically all the muscles of the body except those of the tail, jaws and diaphragm. The temperature drops to subnormal, the animal becomes weaker, and dies within 3 days after becoming prostrate (Amoss 1928). At necropsy, the lesions are similar to those in human beings, and may be briefly summarized as follows: (1) The lesions are chiefly in the grey matter of the cord; the brain is only slightly affected. (2) There is a striking perivascular sheath infiltration, and a more diffuse infiltration of the grey matter of the cord; the cells consist chiefly of lymphocytes, with a fair proportion of polymorphs and a small number of large cells with clear nuclei; plasma cells and lymphoblasts are rare. (3) There is severe degeneration of the ganglion cells of the grey matter; all stages of cellular degeneration, from a slight chromatolysis to complete neuronophagia can be seen. (4) Hemorrhages are usually present in the grey matter of the cord (McIntosh and Scarff 1928, Hurst 1929). Characteristic intranuclear inclusion bodies have been described in the nerve cells of fatal human cases, though in monkeys they are less easy to demonstrate owing to the very rapid necrosis that these cells undergo (Hurst 1931b).

Though Flexner, using *M. rhesus* monkeys, was unable to set up the disease by feeding, Kling, Levaditi and Hornus (1934) showed that *M. cynomolgus* monkeys were susceptible. The results of these workers were confirmed by Lépine and Sédallian (1939) and Vignec, Paul and Trask (1939). Chimpanzees can likewise be infected by the mouth, even after bilateral section of the olfactory tract (Howe and Bodian 1941a). Aisenberg and Grubb (1943) recorded the successful production of poliomyelitis in *rhesus* monkeys by instillation of infective material into the pulp canal of three anterior teeth. The cavities were immediately sealed to prevent escape of the virus. After 8 days all four legs were paralysed. At post-mortem, lesions were found in the Gasserian ganglions, suggestive of a direct nervous spread to the brain.

Armstrong (1939) showed that the Lansing strain of poliomyelitis virus, which had been passed through monkeys, could be transmitted by intracerebral inoculation to cotton-rats (*Sigmodon hispidus hispidus*); and Jungeblut and Sanders (1940) showed that, after becoming adapted to the cotton-rat, the strain could be passed on to white mice. The incubation period in cotton-rats is 3-8 days, in white mice 2-10 days. Paralysis of one or more legs is first observed and death occurs from respiratory failure in 24-48 hours. Recovery is uncommon (Armstrong 1941).

Properties of the Virus.—Flexner and Noguchi (1913) described the cultivation of minute globoid bodies, 0.15-0.3 μ in diameter, in an ascitic fluid rabbit kidney medium, and brought evidence to show that these bodies constituted the causative agent of the disease. The balance of evidence, however, is definitely against this conclusion (see Fairbrother 1929). Later work showed that the virus is probably much smaller—12-17 m μ (Theiler and Bauer 1934), 8-12 m μ (Elford *et al.* 1935). It resisted cultivation for a long time. In 1949, however, Enders, Weller and Robbins succeeded in growing the Lansing strain in cultures of human embryonic tissues, and in demonstrating the extremely interesting fact that growth could occur in tissue devoid of intact neurons. This work opened up a new phase in the investigation of poliomyelitis. Numerous papers by different workers appeared in rapid succession in which it was shown that culture was possible in the presence of human foreskin, human testicle, and later monkey testicle; that the Brunhilde and the Leon types could be grown as well as the Lansing type; that roller-tube cultures in which growth of the virus could be detected by its cytotoxic effect on the out-growing fibroblasts were peculiarly valuable; that colonies of the virus could be demonstrated by the formation of plaques on a layer of monkey kidney cells covered with agar, that tissue culture could be used for primary isolation of the

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The physiological economy of the cell is dynamic rather than static, and life is compatible only with a state of greater or lesser, but nevertheless continuous, physiological activity. This activity may be approximately separated into two phases, the exothermic, oxidative, energy-yielding reactions, and the endothermic reduction reactions of synthesis. The former make possible a continuous, regulated supply of energy to the cell, while the latter channel this energy, or a part of it, into the synthesis of cell substance. The living cell may, therefore, be regarded as a machine for the manufacture of protoplasm, operating with a greater or lesser degree of efficiency, whose energy is supplied by the complex process of respiration. This oxidation-reduction process is not complete within itself, *i.e.*, the machine is not 100 per cent efficient, and the respiratory oxidations are not precisely balanced by the reductions of synthesis. Other substances must, therefore, be reduced to balance the excess of oxidation reactions and this is carried out through a series of reactions leading to the eventual reduction of atmospheric oxygen. The separation of the processes of respiration and synthesis cannot be complete, of course, for in many instances

intensive study of the pathogenesis of the disease likewise showed that antibodies appeared in the blood serum, probably in response to a systemic infection, during the earliest stages of the illness before paralysis had set in. Observations, mainly on antibodies to the Lansing type of virus, revealed the presence of maternally transmitted antibodies in a high proportion of new-born infants. They usually fall to a low level by the end of the 1st year of life and then rise again as the child gets older.

In Baltimore, for instance, neutralizing antibodies were found in 72 per cent. of infants under 3 months of age. This proportion fell to 10 per cent. at 1 year of age, and then rose progressively to 49 per cent. at 4 years, 72 per cent. at 5-9, 84 per cent. at 10-14, and 89 per cent. over 15 years of age (Turner *et al* 1950). Similar observations were made in Cairo, though the rise was more rapid, 90 per cent. of sera containing antibodies by the age of four (Goldblum and Melnick 1952).

Generally speaking, the more primitive the sanitary conditions, the earlier and more widespread is the development of antibodies. Thus Hammon, Sather and Paul (1951), from children in different parts of the world, found antibodies first in the Pacific Islands, slightly later in Japan, then in the United States of America, and latest of all in California. As a rule antibodies develop as the result of a latent infection, and appear most often during the epidemic season. Turner and his colleagues (1950) in Baltimore made repeated tests on 272 children whose serum was free from antibodies at the first examination. Over a 2-year period sera from 51 of these children became positive, indicating a mean annual infection rate of 20.4 per cent. Of 201 initially negative children observed over the summer months approximately 20 per cent. became positive, whereas of 224 children observed through two winters less than 0.5 per cent. became positive. None of the children in whom antibodies developed showed any clinical illness suggestive of poliomyelitis. There is evidence that neutralizing antibodies persist indefinitely in the serum, though complement-fixing antibodies are transient and are indicative of recent infection (Goldblum and Melnick 1952).

Paul, Riordan and Melnick (1951), for example, studying the distribution of neutralizing antibody against different types of poliomyelitis virus in the North Alaskan Eskimos, found neutralizing antibodies of the Lansing type in persons over 20 years of age, and of the Brunhilde and Leon types in persons of over 30 and 40 years of age respectively. They concluded that the presence of these different antibodies was probably associated with epidemics of poliomyelitis in the years 1905, 1915, and 1930, each being due to a different type of virus.

Most of the evidence suggests that neutralizing antibodies protect specifically against clinical disease caused by the homologous type of virus. Whether they protect also against latent infection is more questionable. Hammon (1949), whose review on immunity in poliomyelitis repays careful study, quotes several observations in which the adult members of a family in which a single case of poliomyelitis had occurred became transient throat or intestinal carriers of the virus in spite of antibodies in their serum. On the other hand, Brown and Ainslie (1951) found an inverse relationship in family outbreaks between the presence of neutralizing antibodies and the frequency with which virus could be isolated from the faeces. On the whole the evidence suggests that a lasting type-specific immunity develops

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A reasonable amount of information is available with respect to respiration, regarding both the catalysis of the reactions and the intermediary metabolism of the substrates, i.e., the successive reactions involved. On the other hand, the mechanisms of synthesis of the components of protoplasm are almost completely unknown, not only for the bacteria but for other organisms as well. Elucidation of the nutritive requirements of bacteria may, perhaps, be regarded as definition of the precursors of protoplasm in the catalytic system that is the microorganism under consideration; it may equally well be regarded as definition of the limits of the synthetic abilities of the organism.

RESPIRATION³

Lavoisier's classic experiments on animal respiration performed at the close of the eighteenth century led to the definition of respiration as the utilization of oxygen and the production of carbon dioxide and water, and the presence of molecular oxygen was considered to be absolutely essential for life. However, the meaning of the term respiration was gradually broadened after Pasteur discovered in 1861 that many bacteria can grow and thrive in the complete absence of oxygen. Perhaps the most general definition one can give is that of respiration as the sum total of the chemical reactions carried out by the living cell which result in the liberation of energy.

Of these energy-yielding reactions by far the most important are those of oxidation-reduction. In an oxidation-reduction reaction, one substance is oxidized and one substance is reduced. According to Clark⁴ and to Michaelis,⁵ the oxidized substance loses electrons and the reduced substance gains electrons. If, in the respiration of a living cell, molecular oxygen is the ultimate oxidizing agent, and thus the ultimate acceptor of electrons, the respiration is said to be aerobic. If another substance, not oxygen, is the final electron acceptor, the respiration is said to be anaerobic. The oxidation-reduction reactions of respiration have two functions: they transform nutrient material into substances needed for the maintenance and growth of the cell, and they liberate energy from the nutrient material for use in the cellular economy.

³ For a general discussion of respiration, see Green *Mechanisms of Biological Oxidation*, Cambridge University Press, Cambridge, 1940, and *A Symposium on Respiratory Enzymes*, University of Wisconsin Press, Madison, 1942. The general subject of bacterial respiration has been discussed by Werkman: *Bact. Rev.*, 1939, 3:187.

⁴ Clark, *Public Health Repts.*, 1923, 38:443. This and nine additional papers on oxidation-reduction studies are contained in *Hygienic Laboratory Bulletin* No. 151, 1928.

⁵ Michaelis and Schubert, *Chem. Rev.*, 1938, 22:437.

chemoprophylaxis of the disease. These workers brought evidence to suggest that the application to the nasal mucosa of a 2-4 per cent. solution of alum, or better of a 0.32-0.64 per cent. solution of picric acid, was capable of increasing the local resistance of monkeys to intranasal infection with the virus. Schultz and Gebhardt (1937) found that a 0.5 per cent. solution of zinc sulphate was even more effective. Field trials, however, on human beings in the United States and Canada have been (Tisdall *et al.* 1937, Schultz and Gebhardt 1942). Not

prophylactic effect, but they may do considerable local damage by causing extensive coagulation necrosis of the olfactory epithelium. Moreover, since there is serious doubt as to whether infection in man occurs by the nose, the rationale of this form of prophylaxis rests on very slender grounds. The method has now been generally abandoned.

A curious phenomenon, which may or may not prove to be of value in practice, was observed by Sanders and his colleagues (1953), who found that the subcutaneous injection of detoxified cobra venom (from *Naja flava*) 24 hours after intracerebral inoculation of monkeys with poliomyelitis virus delayed the onset of the disease.

Vaccination.—Since neither eradication of the source of infection nor successful interference with the transmission of infection appears to be practicable, it is logical to attempt to control the disease by raising the resistance of the host. Early attempts to vaccinate children were made by Kolmer and his colleagues (Kolmer and Rule 1934a, b, Kolmer 1935, Kolmer *et al.* 1935), using a 4 per cent. suspension of infected monkey cord in 1 per cent. sodium ricinoleate. The virus, though living, was said to be attenuated. Brodie (1934b) and Brodie and Park (1935) recommended a vaccine in which the virus had been inactivated by exposure to 0.1 per cent. formol at 37° C for 8-12 hours. Both of these vaccines were tried on a small scale in the United States. The demonstration, however, by Olitsky and Cox (1936) that Kolmer's vaccine might occasionally give rise to poliomyelitis in monkeys, and the even more serious development of the disease in children, occurring apparently as the result of prophylactic vaccination (Leake 1935), led to the discontinuance of their use.

These unfavourable results set back the clock for the time being, but investigation was renewed and enormously stimulated when evidence accumulated to show the probable occurrence of an early viraemic stage in the disease, and when the way of growing the three different types of virus in tissue culture was discovered. Morgan, Howe and Bodian (1947) brought evidence to show that monkeys could be protected against intracerebral inoculation by intramuscular injections of a vaccine inactivated by formalin or ultraviolet irradiation, and that the degree of resistance was related to the height of the antibody content of the serum. Morgan (1949) obtained evidence that immunity of monkeys following vaccination was probably type-specific. Later, Melnick and Ledinko (1951) found that, with *cynomolgus* monkeys infected by the mouth with living virus of the Lansing type, animals that failed to develop paralysis were subsequently resistant to infection by the intranasal or intracerebral route and that the degree of resistance was associated with the antibody content of the serum. In 1953 Salk reported the first major trial on children. The vaccines he used were prepared from the three different types of virus grown in roller-tube cultures of monkey kidney. Inactivation was carried out by exposure to 0.4 per cent. formalin at 1° C. over a period of several days, the action of the formalin being finally neutralized by sodium bisulphite. The product was stored in the refrigerator. No vaccine was used till it was shown to be innocuous when injected intracerebrally into monkeys. Aqueous vaccines injected intradermally into human subjects were found to stimulate the

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Infections due to the Coxsackie Group of Viruses

In 1918 Dalldorf and Sickles isolated from the stools of 2 children suffering from poliomyelitis a virus that induced paralysis in suckling mice and hamsters but had no effect on *rhesus* monkeys. Paralysis followed intracerebral, intraperitoneal, or intramuscular injection and was characterized by widespread degenerative lesions of the skeletal muscles with little apparent effect on the central nervous system. The following year Dalldorf (1919) reported the isolation of 10 further strains and noted that they were not all antigenically alike. Though a rise in the neutralizing antibody content of the serum was observed in some of the patients, the part played by the virus in the causation of the clinical disease remained doubtful. Widespread interest was aroused by the descriptions of the new virus and it was not long before numerous other strains were isolated in the United States (Curnen *et al.* 1919, Melnick *et al.* 1950, Huebner *et al.* 1950, Weller *et al.* 1950), in Canada (Rhodes *et al.* 1950a), in Great Britain (Findlay and Howard 1950), and in other countries (see Vivell and Gudeke 1952). From the town in New York State in which it was first isolated, it was called the Coxsackie virus.

The virus is found mainly in faeces and sewage, less often in the throat. It has also been isolated from flies. It is spherical in shape. Estimates of its diameter range from 6 $m\mu$ to 37 $m\mu$ according to the strain examined and the method used. It is difficult to propagate in the laboratory, and only occasional strains have been adapted to growth in the fertile egg or in tissue culture. In mouse brain suspension and in faeces it remains stable at -70° C. for several months. It is killed by heat at 55° C. within 30 minutes. It survives in 50 per cent. glycerol for at least 5 months, but is inactivated at room temperature by 0.25 per cent. formalin (Dalldorf *et al.* 1919). It is destroyed by 0.6 p.p.m. of chlorine at pH 7.0 in about 10 minutes (Clarke and Kabler 1951). By complement-fixation and neutralization tests at least 16 different antigenic types can be distinguished (see Contreras *et al.* 1952).

According to the lesions they give rise to in suckling mice the different antigenic types are classified into two groups—A and B. Strains of Group A injected intracerebrally into suckling mice lead to generalized weakness in 2–3 days followed by death or paralysis the following day. At post-mortem the skeletal muscles are white and firm in appearance, and microscopically show hyaline degeneration of the kind described by Zenker. Strains of Group B, on the other hand, cause either spasticity or paralysis with delayed death. The irregular tonic contractions of the muscles may give rise to torsion of the body resulting in what is sometimes known as the *rolling disease*. At post-mortem there are found focal myositis, softening of the brain, and necrosis of the subcutaneous and visceral fat, some strains may cause hepatitis or massive necrosis of the acinar tissue in the pancreas (Dalldorf 1950, Pappenheimer *et al.* 1951). The distinction between the two groups is not always clear. There is a certain amount of overlapping, and occasional strains resemble viruses of the encephalomyocarditis group (see p. 2155). The distinction between them may be aided by the circumstance that in their growth in tissue cultures the viruses of Groups A and B are said to differ in their nutritional requirements (Weller *et al.* 1953). A third group, called Group C, was described by Stanley, Dorman and Ponsford (1953) in association with an outbreak of encephalitis at Sydney. It differed from Groups A and B in various respects, notably its lack of virulence for mice more than 24 hours old and its failure to affect the skeletal muscles.

Bacterial Physiology

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lasting 2-5 days or so have been reported in children and adults during the summer months. The chief clinical features are usually fever, headache, sore throat, and myalgic pains in the back, neck, abdomen, and limbs. There is, however, considerable variation in the symptomatology, and a tendency to merge into the picture of aseptic meningitis. In some of these outbreaks Cocksackie virus has been demonstrated in the stools of a high proportion of the patients examined and neutralizing antibodies have appeared in the serum during the course of the disease (Melnick *et al.* 1950, Kenyon *et al.* 1952, Wood *et al.* 1952).

Bornholm disease : epidemic myalgia : epidemic pleurodynia.—This disease, which has been observed in various parts of the world, was described by the Danish physician Sylvest, who studied an outbreak in Bornholm in 1930 and published a monograph on it in 1931. It is characterized by fever, acute pain, usually of sudden onset, affecting the chest, upper abdomen, or lower region of the back on one or both sides, often headache, little or no constitutional disturbance, an absence of catarrh, and a tendency to relapse. The pain is paroxysmal and lasts for a day or more. The muscles are tender on pressure. Relapses are common at intervals of 2 or 3 days, or after a longer period. Orchitis may be seen as a complication. Fatal cases are rare. The incubation period is 2-4 days. The disease occurs in epidemic form, usually in the late summer. Infection probably spreads by contact and is caused by the B type.

A similar disease, differing in not affecting the respiratory muscles of the chest and abdomen and in being comparatively afebrile, was described by Beeson and Scott (1942) under the term *epidemic myalgia of the neck and shoulders*.

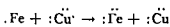
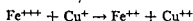
In several outbreaks of the Bornholm type Cocksackie virus has been isolated from the throat or faeces of a considerable proportion of the patients examined, and the development of antibodies demonstrated in the blood (Weller *et al.* 1950, Findlay and Howard 1950, Lazarus *et al.* 1952, Bury and Tobin 1952, Gabinus *et al.* 1952). In 2 cases studied by Lépine, Desse and Sautter (1952) lesions were demonstrated in the muscles similar to those found in suckling mice, and the Cocksackie virus was isolated from both the muscles and the faeces.

It may be added that in *diagnosis* of the Cocksackie group of infections great care must be exercised in the interpretation of the serological reactions. Antibodies to various types of the virus are often present in normal persons. Complement-fixing antibodies are not strictly type-specific and the titre to heterologous types may be higher than that to the homologous type (Kraft and Melnick 1952). For fuller information on this virus and the diseases it causes the reader may consult the reviews by Melnick and Curnen (1952), Vivell and Gadeke (1952), and Tobin (1953).

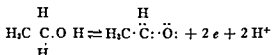
RABIES

Rabies is a disease of animals affecting particularly dogs, cats and carnivorous wild animals; herbivora are less frequently attacked. The disease is transmissible to human beings by the bites of rabid animals, notably dogs, cats, wolves, and jackals. The incubation period varies according to the age of the animal, the situation of the bite, and the severity and nature of the bite. Generally speaking, it varies in animals from 2 to 8 weeks, and in human beings from 6 to 8 weeks; it tends to be shorter in young animals and children, and after severe lacerated or

Oxidation-Reduction Potential.⁶ A simple example of an oxidation-reduction reaction is the reaction between ferric iron and cuprous copper.



When the oxidation-reduction involves organic compounds, as in almost all biological oxidations, oxidation often results in transfer of hydrogen atoms as well as of electrons, and biological oxidation is often referred to as hydrogen transport. The oxidation of ethanol to acetaldehyde, for example, is accompanied by transfer of hydrogen as well as of electrons.



It is obvious that any substance capable of entering into oxidation-reduction reactions must exist in two forms—an oxidized form and a reduced form. These two forms constitute a reversible oxidation-reduction system.



Oxidized form Reduced form

The oxidizing power of an oxidation-reduction system is a function of its ability to donate electrons to another system and may be quantitatively expressed as the oxidation-reduction potential of the system.

The oxidation-reduction potential has the dimensions of volts and may be measured in a suitable electrical circuit. The oxidation-reduction potential, E_h , is defined by the general electrode equation,

$$E_h = E_o - \frac{RT}{nF} \ln \frac{[\text{Red}]}{[\text{Ox}]}$$

in which E_h and E_o are measured in volts, R is the gas constant, T is the absolute temperature, F is the faraday, and n is the number of electrons transferred in the oxidation-reduction reaction. The observed oxidation-reduction potential, E_h , is a function of E_o , a constant for the system, and the ratio of the molar concentrations of the oxidized and reduced forms. When

$$\frac{[\text{Red}]}{[\text{Ox}]} = 1, \quad \text{then} \quad E_h = E_o$$

and this relationship serves to define the constant, E_o . Each oxidation reduction system has a characteristic E_o value. The potential of the system,



at pH 0 and 1 atmosphere of hydrogen is arbitrarily assumed to be 0, and all other oxidation-reduction potentials are expressed in relation to the potential of this system, the normal hydrogen electrode.

If either or both the oxidized or reduced forms of a system are ionized, changes in pH will alter the potential of the system by disturbing ionic equilibria. Because of this effect the pH must be specified for each observed value of E_h , or else the value is meaningless. The E_o of a system represents the potential at which the system is half-oxidized at pH 0, and another symbol, E_o' , is used to represent the potential of a half-oxidized system at a specified pH.

⁶ For a general discussion appropriate here, see Hewitt: *Oxidation-Reduction Potentials in Bacteriology and Biochemistry*. 4th ed. London County Council. P. S. King & Son, Ltd., London. 1936.

injected subcutaneously, the street virus alone proves virulent. It would appear that by passage through the rabbit's brain, the fixed virus has acquired a strong neurotropic affinity, which enables it to give rise to rabies in 7 days after subdural injection, but which usually renders it avirulent on subcutaneous inoculation. The street virus, on the other hand, takes about 14 days to produce rabies on subdural inoculation, and usually proves virulent when introduced subcutaneously. Fixed viruses can likewise be produced by passage through guinea-pigs, dogs, hens, and monkeys (Pasteur *et al.* 1884). Both street viruses, and the fixed viruses derived from them, show considerable variability in their virulence for animals; some rarely produce infection when inoculated subcutaneously, others always do so (Marie *et al.* 1927). Pasteur (Pasteur *et al.* 1884) found that by passage through the brain of monkeys, the virus diminished in virulence for dogs, though when passed through rabbits or guinea-pigs it uniformly increased in virulence.

The virus is present in the saliva of rabid animals, and has sometimes been demonstrated in it 4 days before the onset of clinical symptoms. It is also found in the central nervous system, the peripheral nerves, and in the salivary glands, less regularly in the cerebrospinal fluid. Occasionally it may be demonstrated in the blood and the internal organs (Pasteur *et al.* 1884, Remlinger and Bailly 1931, 1932). The virus passes along the nerves from the local injury, and reaches the central nervous system; this is shown by the fact that (1) the animal may live after resection of experimentally infected nerves; (2) the resected nerves are themselves infective; (3) the portion of the spinal cord in connection with the infected nerve becomes infective before other parts of the central nervous system (di Vestea and Zagari 1889, Marie *et al.* 1927). According to Nicolau and Galloway (1928), the virus, when introduced into the brain, may spread centrifugally, and be found in such nerves as the brachial and sciatic; the passage of the virus along the nerves sets up an interstitial neuritis. It has been suggested that the salivary glands owe their infectivity to the presence in them of neurones, which may occur as single cells or as ganglionic aggregations, and which are situated just under the epithelium; if the epithelium is abraded, the virus is set free from the neurones, and infects the saliva (Manouélian and Viala 1927, 1928; for references see McKendrick 1928).

Properties of the Virus.—The rabies virus can pass through Berkefeld candles. Galloway and Elford (1936), from filtration experiments carried out with gradocol membranes on a strain of fixed virus, estimate its size as 100–150 μ . This agrees with the value obtained for another strain of fixed virus by Yaoi, Kanazawa and Sato (1936). The virus can be grown in tissue cultures containing embryo brain and human or monkey serum (Kanazawa 1936, Webster and Clow 1937). It can also be cultivated on the chorio-allantoic membrane of the developing chick embryo, provided 5- or 6-day embryos are used (Kligler and Bernkopf 1941). Cultivated strains tend to lose their virulence fairly rapidly. According to Sankaran, Iyengar and Beer (1934), the virus can be separated from infected rabbit brain by electrophoresis. It is not very resistant. It is destroyed by exposure to a temperature of 60° C. for 30 minutes. Slow drying gradually renders it inert, but if rapidly dried it may retain its infectivity for months. The fixed virus is very sensitive to the photodynamic action of methylene blue (Shortt and Brooks 1934, Galloway 1934) and to irradiation with ultraviolet light (Sankaran and Beer 1935). It is readily killed by bile. Little is known about its antigenic structure, but most strains seem to resemble each other fairly closely (Remlinger

Systems with relatively high E_0 's have strong tendencies to accept electrons and are good oxidizing agents. Systems with relatively low E_0 's have strong tendencies to donate electrons and are good reducing agents. The oxidized form of any system may oxidize (accept electrons from) the reduced form of any system with sufficiently lower E_0 . Such reactions always proceed with the liberation of energy (decrease in the free energy of the system). That is, the energy contained in the products of the reaction is always less than that contained in the reactants.

Application to Biological Systems. The foregoing treatment of oxidation-reduction potential applies only to completely reversible systems. A number of biological systems are reversible and electromotively active and therefore susceptible to such analysis. This group includes the cytochromes and cytochrome oxidase, the flavoproteins, and a number of naturally occurring pigments. A second group is made up of the sluggish systems, so called because of their sluggish behavior at the electrode, which are regarded as only partially electromotively active. The pyridine nucleotides and the sulfhydryl compounds such as glutathione and cysteine belong to this group, and the measurement of their oxidation-reduction potentials is difficult and generally unsatisfactory. Lastly, there are those sluggish systems that develop potentials only in the presence of specific enzymes, the enzymatic oxidation-reduction systems. There are many of these, including succinic acid-fumaric acid, lactic acid-pyruvic acid, alcohol-acetaldehyde, and others.

Since many of the oxidation-reduction systems in the living cell fall into this last group, it is very difficult to interpret potentials produced during active cellular metabolism when a number of systems must be functioning simultaneously. As a matter of fact, it is doubtful that the reducing intensities developed should be termed "oxidation-reduction potentials" at all. Probably reduction potential or reducing intensity is the most satisfactory term for potentials developed by the actively metabolizing intact cell. The accompanying table gives the oxidation-reduction potentials of some important biological systems.

OXIDATION-REDUCTION POTENTIALS OF BIOLOGICALLY IMPORTANT SYSTEMS AT pH 7

Oxidation-Reduction System		E_0 in volts	T C*
Oxidized Form	Reduced Form		
O_2	H_2O	0.81	25
Ferro-cytochrome oxidase	Ferro-cytochrome oxidase	?	
Ferro-cytochrome <i>a</i>	Ferro-cytochrome <i>a</i>	0.29	25
Ferro-cytochrome <i>c</i>	Ferro-cytochrome <i>c</i>	0.26	25
Ferro-cytochrome <i>b</i>	Ferro-cytochrome <i>b</i>	-0.04	25
NO_3^-	NO_2^-	0.05	30
Methylene blue	Reduced methylene blue	0.01	30
Pyocyanine	Reduced pyocyanine	-0.03	30
Fumaric acid	Succinic acid	-0.03	25
Flavoprotein	Reduced flavoprotein	-0.06	38
Oxalacetic acid	Malic acid	-0.10	37
Phthiocol	Reduced phthiocol	-0.17	30
Pyruvic acid	Lactic acid	-0.18	35
Acetaldehyde	Ethanol	-0.19	30
Diphosphopyridine nucleotide	Reduced diphosphopyridine nucleotide	-0.29	30
H^+	H_2	-0.41	25

the mouse passage method (Webster and Dawson 1935), affords the most rapid means of determining the presence of infection in a suspected animal.

Diagnosis.—In attempting to reach a diagnosis of rabies in a human being before the disease has declared itself, it is important if possible, not to kill the animal responsible for the bite, but to keep it in safe captivity for a few days to see whether the typical symptoms of the disease develop. If it is killed before the onset of the paralytic stage, no Negri bodies may be found in the brain. Once the animal is paralysed, it should be killed, and the brain examined by a rapid section method for Negri corpuscles, or preferably by impression smears stained by Sellers' method (see Johnson 1952). Negri corpuscles are demonstrable in only about 90 per cent. of animals and 70 per cent. of human patients that have died of rabies. Resort should therefore be had to the mouse inoculation test described by Webster and Dawson (1935) if the brain of a suspected animal fails to reveal these bodies microscopically. The mouse test should also be used when the animal has been prematurely destroyed or the brain improperly preserved.

Webster (1936) recommends that sterile pieces of Ammon's horn of the suspected animal should be made into a 5 or 10 per cent. suspension with distilled water, and that 0.03 ml. should be inoculated intracerebrally and 0.25 ml. intramuscularly into each of 6 mice belonging to the specially susceptible Swiss breed. One animal should be killed on the 5th, 6th, and 7th days respectively, and its brain examined for Negri bodies. The remaining three are observed for 4 weeks for signs of rabies. In practice, a negative diagnosis may usually be given in 3 weeks (Thomas 1939). The advantage of the mouse over the rabbit is that it is more susceptible, it is easier to inoculate intracerebrally, and it develops rabies, on the average, a week earlier.

If the brain of a suspected animal cannot be examined fresh, it should be packed in ice before transmission to the laboratory; or, alternatively, half of it may be sent in 10 per cent. formalin for section and half in 50 per cent. glycerol for inoculation.

An attempt may be made to isolate the virus from the saliva of man or animals, particularly in cases of the paralytic type, which often present considerable difficulty in diagnosis. For this purpose intramuscular injection of the Syrian hamster is to be recommended, as this animal is very susceptible to street virus given peripherally (Koprowski 1949). Complement-fixation and neutralization tests may be used to demonstrate a rise in the patient's serum antibodies and are recommended as a routine procedure by Johnson (1949). In practice, however, they are seldom of value, nor are they suitable for diagnosis of the disease in animals (Terkel 1948).

Prophylactic Treatment of Rabies.

After infliction of the bite, an attempt should be made to destroy as much of the virus in the wound as possible. For this purpose, treatment with a 20 per cent. solution of soft soap is said to be as effective as cauterization with fuming nitric acid (Shaughnessy and Zichis 1943). Tincture of iodine is also useful, if applied within 30 minutes.

Vaccination.—Rabies is a fatal disease, and once symptoms have declared themselves, treatment is of no avail. But the disease is characterized by a long incubation period; and it was Pasteur (1885) who realized the possibility of using this period for carrying out specific vaccine treatment. Pasteur found that the spinal cords of rabbits dying of experimental rabies gradually lost their virulence when dried in air over KOH. By inoculating dogs with cords dried for varying

Reduction Potentials of Bacteria. The development of reducing properties by bacteria is associated with equivalent electrometric and colorimetric changes, indicating that the predominant system or systems are reversible. The potentials may be developed by bacterial suspensions in the presence of added substrate, or during growth in culture; the latter state has been by far the more commonly studied.

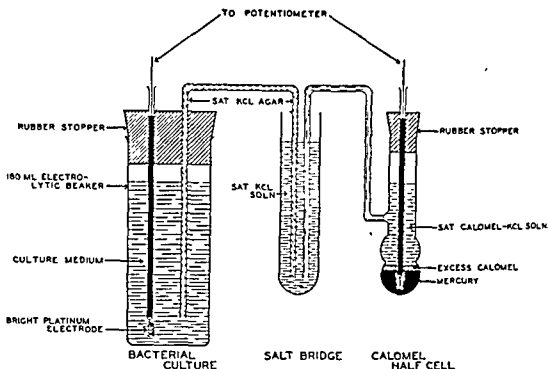


Fig. 17. Diagrammatic representation of the method used in the measurement of reduction potentials developed in bacterial cultures. The culture vessel should contain duplicate electrodes and an additional opening for inoculation, removal of samples, etc.

Measurements of the reducing intensity are made electrometrically, using bright platinum or gold plated platinum electrodes, as the use of indicator dyes which change color with oxidation or reduction has been unsatisfactory. A satisfactory arrangement is illustrated in Fig. 17 in diagrammatic form. The bacterial culture is a half cell, and the standard half cell is a saturated calomel cell. The whole is immersed in a constant temperature bath. With buffered broth as the culture medium, the pH is maintained without significant variation. The reducing capacity of the bacterial culture is very low, *i.e.*, it is poorly poised (analogous to poorly buffered with respect to changes in pH), and if appreciable amounts of current are passed through the system in the measurement of the potential, the electrodes polarize, making readings impossible. It is necessary, therefore, to insert a high resistance in series with the cell, so that only a very small current passes through the culture. The calculation of the potential is simple:

$$E_h = E_{obs} + E_{std}$$

where E_{obs} is the observed potential and E_{std} is the potential of the standard half cell. Measurement of the developing potential may be made at intervals to give a time-potential curve.

The potential of a freshly-prepared sterile culture medium often shows a slight, gradual negative drift. When inoculated with bacteria, however, the

time for them to appear is between the 11th and 30th days after the bite, that is to say usually before the onset of rabies in untreated persons. The severer forms of paralysis prove fatal in about 25 per cent. of cases. What they are due to is not known with certainty, but there is reason to believe that, like the encephalitis following some of the infectious diseases, they are allergic in nature (see Remlinger 1927, Hurst 1952).

The average case fatality in treated patients in McKendrick's series varied over a number of years between 0.23 and 0.49 per cent.

How, it may be asked, can a dead vaccine, inoculated into the patient after living virulent virus has gained access to the body, afford any protection against the disease? The virus is known to be neurotropic, and almost certainly finds its way to the brain and cord by way of the nerves. If an attenuated virus was inoculated in enormous quantities very soon after the bite, it is conceivable that some of it might reach the susceptible nerve cells and block the entry of the virulent virus. But how can this happen with a dead vaccine, unless transport along the neurones is purely mechanical? Yet, from McKendrick's figures a dead vaccine appears to be just as efficacious as a living attenuated virus. The only answer to this question is to assume that the vaccine reaches the central nervous system by the blood stream and there causes the necessary interference. Surprisingly enough, there seems to be no advantage in early treatment; the fatality of those treated 0-4, 5-7, or 8-14 days after the bite was, among Europeans, 0.19, 0.10 and 0.06 per cent. respectively. Animal experiments do not increase our confidence in vaccination. The observations of Shortt and of Covell and their colleagues in India (Shortt *et al.* 1934, 1935, Covell *et al.* 1936), and of other workers (see Webster 1939*b*) seem to show that (1) no vaccine can protect an animal *after* exposure to infection by rabies virus, (2) vaccine given *before* exposure to infection is effective only provided (a) it is given repeatedly and in large doses, totalling at least 1 per cent. of the body weight, and (b) the test dose is given subcutaneously, intramuscularly, or by some other route than the intracerebral.

Considerations such as these have led many workers to question the value of the vaccine treatment of rabies. Ever since Pasteur recorded his apparently brilliant successes, persons who have been bitten by rabid animals have been given treatment as a matter of course. In such a disease it would be unjustifiable to withhold it so long as there was any reason to believe that it might confer some degree of protection. The result has been that there are no adequate control figures available. A high proportion of persons treated have probably not been bitten by rabid animals, and, of those who have, probably not more than a quarter would have developed rabies anyway. The figures of the Pasteur Institutes therefore throw little light on the main question at issue.

Progress in the past has been hampered by the absence of a satisfactory technique for standardizing anti-rabic vaccines. Webster (1939*a*) filled this need by introducing a mouse inoculation test. By its use he found that commercial vaccines of the killed type were often devoid of protective action or were effective only in doses greater than those recommended by the manufacturers (see also Wyckoff and Beck 1940). Webster's technique was modified by Habel (1940*a*), whose mouse potency test is the one now generally adopted. Comparative titrations of different vaccines revealed considerable variations in the antigenic potency of the strains used to prepare commercial vaccines (Habel 1940*b*), and the results of the potency tests with any one vaccine varied according to the strain of infecting virus

potential falls rapidly during the early hours of incubation. Thereafter, it differs somewhat with the type of bacterium. In the case of the obligate anaerobes (see Fig. 18) it reaches and maintains a very low level, sometimes approaching or reaching the level of hydrogen overvoltage, i.e., more negative than the normal hydrogen electrode. Some facultative anaerobes such as the colon bacillus reach similar low levels while others do not. Other kinds of bacteria behave still differently. *Pneumococcus* cultures, for example, show a rapid positive drift after a negative potential has been established, this is probably due to the accumulation of peroxide since it does not occur in catalase broth cultures. Representative time-potential curves are given in Fig. 18.

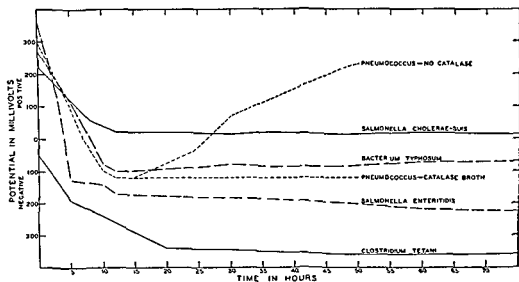


Fig. 18 Time potential curves illustrating the development of reducing intensities in bacterial cultures. Note the rise in potential in pneumococcus cultures in non-catalase containing media resulting from the accumulation of peroxide. The development of species-characteristic potentials is apparent. *Pneumococcus* data from Hewitt, *Cl. tetani* from Gillespie and Rettger, remainder from Burrows and Jordan.

Mechanism of Biological Oxidation.⁷ The importance of hydrogen transfer in biological oxidations was early recognized and particularly emphasized in the work of Thunberg and Wieland. Wieland postulated that in biological oxidation-reductions the hydrogen atoms of substrates were activated by specific enzymes called dehydrogenases, once activated, these hydrogen atoms were then spontaneously transferred to any suitable hydrogen acceptor. However, at about the same time, Warburg showed that oxygen may be similarly activated by an iron-porphyrin enzyme (*Atmungsferment*) and postulated that activated oxygen could spontaneously accept hydrogen from substrate molecules. The ideas of Wieland and Warburg were soon harmoniously reconciled in the now generally accepted view of biological oxidation in which substrate hydrogen is activated by dehydrogenases and molecular oxygen

⁷ In addition to the general references given in 3, see also Goddard's article "The Respiration of Cells and Tissues" in Hober: *Physical Chemistry of Cells and Tissues*. The Blakiston Company, Philadelphia, 1945, and Oppenheimer and Stern. *Biological Oxidation*. Nordemann Publishing Co., Inc., New York, 1939.

only antibodies to a somatic antigen are protective (see Chapter 33). Burrows (1953) notes a more subtle distinction of this kind with *V. cholera*; the complete O antigen is a better immunizing agent than the endotoxin against asymptomatic infection of the intestine in the guinea-pig; whereas endotoxin effectively immunizes mice against the predominantly toxic infection that follows intraperitoneal injection of the living vibrio.

It was, however, in the case of the motile bacilli, possessing flagellar and somatic antigens, that the problem of the different immunizing value of different bacterial antigens was first stated in its clearest and most explicit form by Felix and his colleagues (see Felix 1924). The results of subsequent studies strongly support his contention that the flagellar antigens, and their corresponding antibodies, play little if any part in specific antibacterial immunity, whereas the heat-stable somatic antigens and their corresponding antibodies are all-important.

Arkwright (1927) immunized guinea-pigs by the injection of vaccines prepared from different strains of *Salm. typhi* and *Salm. paratyphi A*, and subsequently tested their resistance by the injection of living virulent bacilli.

With *Salm. typhi*, a vaccine prepared by the use of a smooth, motile, virulent strain (containing the flagellar antigen and the surface antigen IX) produced a significant increase in resistance. A vaccine prepared from a rough, motile variant (containing the flagellar antigen but not the surface antigen IX) did not. With *Salm. paratyphi A* four vaccines were used: (1) a smooth, motile strain, with the flagellar antigen and the surface antigens I, II; (2) a non-motile, smooth variant, with no flagellar antigen but retaining the surface antigens I, II; (3) a rough, motile variant, with the flagellar antigen but without the surface antigens I, II; and (4) a rough, non-motile variant containing neither the flagellar antigen nor the surface antigens I, II. Vaccines (1) and (2), containing the antigens I, II, produced a marked increase in resistance. Vaccines (3) and (4), from which this antigen was absent, did not.

Ibrahim and Schutze (1928) obtained analogous results in the immunization of mice against *Salm. typhi-murium*. They found that the R variants, in which the specific polysaccharide antigen was absent, were ineffective as immunizing agents. The S variants, containing the specific polysaccharide antigen, produced a significant increase in resistance.

Experiments, in which untreated mice and mice vaccinated with different bacterial suspensions were submitted to the risk of natural infection during a long-continued experimental epidemic of mouse typhoid, also yielded entirely concordant results (Greenwood, Topley and Wilson 1931). The total numbers in each of the vaccinated groups were large—over 300 mice—so that the difference noted may be regarded as certainly significant. The results are summarized in Table 72.

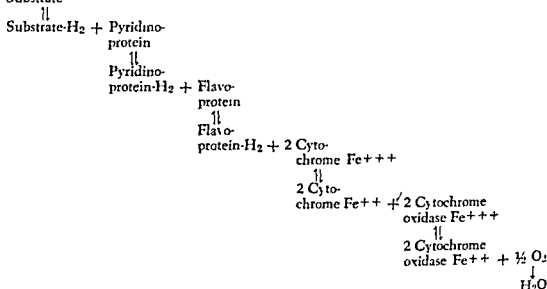
The column giving the antigenic structure shows the components on the surface of the bacterial cells and those carried by the flagella. All vaccines except E were saline suspensions, killed by heat and formalin. Vaccine E—a killed broth culture—was included because of the possibility that some bacterial product of immunizing value might be liberated into the culture fluid. The measure of resistance—the mean survival time in the epidemic cage limited to 60 days—was selected for reasons that will be referred to later. The significance of the standard error, which is attached to each survival time, has been considered in Chapter 43.

It will be seen that the control and vaccinated groups can be divided into three classes on the basis of their survival time. The unvaccinated mice lived, on the average, for 26.26 days, and were thus significantly less resistant than any other group. The groups D, B and A were slightly more resistant. This increased resistance was clearly not specific. The rough polysaccharide antigen was not concerned, for the *Staph. albus* vaccine did not contain it. The flagellar antigens of *Salm. typhi-murium* were not concerned, for neither

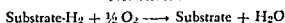
acts as a hydrogen acceptor only after activation by Warburg's respiratory enzyme.⁸ The dehydrogenases of Wieland have been found to consist of a specific enzyme protein and a low molecular weight coenzyme which undergoes reversible oxidation-reduction. The respiratory enzyme of Warburg functions in respiration by catalyzing the oxidation of other iron-porphyrin protein enzymes, the cytochromes, by molecular oxygen, and for this reason has been given the functional name, cytochrome oxidase. Interposed in the train of hydrogen and electron transport between the dehydrogenases and the cytochromes is another type of respiratory enzyme, the flavoprotein, which is reduced by the dehydrogenases and oxidized by the cytochromes. Thus, there is a clear and continuous pathway for hydrogen and electron transport from the substrate to molecular oxygen, which is illustrated in the accompanying diagram.

AEROBIC OXIDATION OF SUBSTRATES

Substrate



Net Reaction



In most aerobic organisms this system is complete and functional, but in facultative and obligate anaerobes, portions of the hydrogen transport chain may be absent or non-functional (*vide infra*). In anaerobic respiration, the hydrogen and electrons of substrates are not transferred to molecular oxygen but to other substrates instead. The iron-porphyrin catalysts are not involved in anaerobic respiration, but the flavoproteins may sometimes act as intermediate carriers.

Respiratory Enzymes in Bacteria.⁹ Although bacteria contain very active respiratory enzymes, much less is known about their chemical nature than about similar enzymes in yeast and in the tissues of higher animals,

⁸ Kluyver and Donker: *Chem. Zelle Gewebe*, 1926, 13:134.

⁹ See Porter: *Bacterial Chemistry and Physiology*. John Wiley and Sons, Inc., New York. 1946. Chapter 6.

but Koprowski and Cox (1951) report on its successful use in 29 patients bitten by dogs that were proved to be rabid. Possibly combined active and passive immunization may prove better than either separately. Experiments made on this point with hamsters were not convincing (Koprowski *et al.* 1950), but later observations on guinea-pigs and dogs yielded more hopeful results (Koprowski and Black 1951). How antiserum acts is doubtful. Presumably it neutralizes the extracellular virus around the bite; if so, it must be given within 2-3 days of the time of infliction of the wound before the virus has gained access to the neurons and passed beyond the reach of the antibody. It should be followed by a 7-day course of vaccine treatment. For much useful information on rabies, see the monograph by Remlinger and Bailly (1938*a*), the article by Johnson (1952), the review by Rhodes (1916) on anti-rabic treatment, and the series of papers on laboratory diagnosis, vaccine production and testing, and the preparation of hyperimmune serum edited in book form by the World Health Organization (Report 1951).

PSEUDORABIES

SYNONYMS: Mad itch: Infectious bulbar paralysis: Aujeszky's disease.

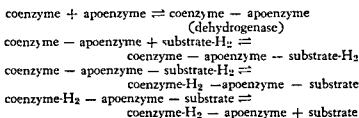
This disease, which was described by Aujeszky in 1902, has only recently attracted much attention. It appears to be an acute infection of the central nervous system affecting dogs, cats, cattle, horses, pigs, goats, sheep, rats, and mice, but not man. Though originally met with in Hungary, it has now been reported from a number of other European countries and from both North and South America. Unlike rabies the incubation period is very short—only a matter of hours—the onset is brusque, there is intense pruritus, which often completely dominates the clinical picture, the mental faculties are preserved, paralysis occurs very late and does not affect the lower jaw, the disease lasts only 21-48 hours, and sudden death is common (Remlinger and Bailly 1931). Except in pigs, the case-fatality rate is usually very high. The mode of infection is still in doubt. According to Shope (1935), the disease in cattle is very fatal but non-contagious, whereas in pigs it is relatively mild but highly contagious. Shope believes that in pigs infection occurs by the nose, and that the abraded skin of cattle can probably be infected from contact with the pig's snout. A study of neutralizing bodies in the serum suggests that pseudorabies is a highly prevalent but unrecognized disease in the hogs of the Middle West. There is some evidence that the disease is spread by rats, and that pigs may become infected by feeding on contaminated material.

The disease can be reproduced experimentally in most animals, though monkeys appear to be fairly resistant. In the rabbit, which is the most susceptible of the small laboratory animals, the disease exhibits a diverse and striking symptomatology. Infection is possible by practically any route. Intracerebral inoculation gives rise in 20-40 hours to a condition of wild excitement, accompanied by salivation, grinding of the teeth, blindness, and later coma and death. In rabbits injected subcutaneously the incubation period is 50-75 hours; the animals then start scratching and biting the site of inoculation; their efforts become increasingly savage till the skin is hairless, abraded, and bleeding; feebleness sets in, and death occurs in a state of collapse 6-24 hours after the onset of pruritus (Shope 1931). The clinical picture, however, is very variable, and may assume an encephalitic, pseudo-herpetic, meningeal, paralytic, pruriginous, fulminating, or even abortive form (Remlinger and Bailly 1934). Some animals can be infected by feeding.

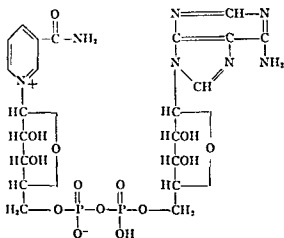
chiefly because it has been very difficult to obtain active enzyme extracts from bacterial cells.

Dehydrogenases Dehydrogenases consist of a specific protein, or apoenzyme, combined with a reversibly oxidized and reduced coenzyme. They oxidize metabolites and are in turn reoxidized by other enzymes, not by molecular oxygen. Dehydrogenases possess a high degree of specificity for the substrate which they oxidize, and are usually named on the basis of their substrate specificity, *i.e.*, lactic dehydrogenase, succinic dehydrogenase, etc. This substrate specificity is a function of the protein component, since a single coenzyme may be a part of several different dehydrogenases

The specific protein combines reversibly with both coenzyme and substrate. The actual oxidation-reduction probably occurs while all three are in physical combination.



Two general types of dehydrogenases are found in bacteria and in other containing either di- or enzymes. The provisional is illustrated



diphosphopyridine nucleotide

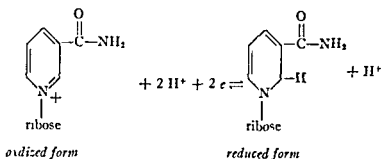
The structure of TPN (coenzyme II) is identical with that of DPN except for the presence of an additional molecule of phosphoric acid which is probably esterified to one of the hydroxyl groups of the pentose residue. Only the pyridine ring of both DPN and TPN undergoes reversible oxidation-reduction as indicated in the accompanying equation.

so-called "trotting" variety, the itching is less severe, but the animal periodically drops every now and again, as if dead, and then rises in a few minutes and resumes its trot. Pathologically, no characteristic lesions have been found, but there is a mild degree of meningo-encephalitis. Early attempts to transmit the disease failed, possibly because the animals were not observed long enough. Later, Cuillé and Chelle (1936, 1938) in France were successful in reproducing the disease in susceptible sheep by subcutaneous, intra-ocular, epidural, and intracerebral inoculation of suspensions of brain and spinal cord. About the same time workers in Scotland (see Gordon 1946, Greig 1950) reported the occurrence of the disease in sheep that had been injected about 2 years previously with a vaccine against louping-ill. This had been made up with a suspension of brain, cord, and spleen from sheep inoculated intracerebrally with louping-ill virus, and had been preserved with 0.35 per cent. formalin. The incubation period of the natural disease appears to be 2 years or longer. After subcutaneous injection of infective material it is usually 15 months or more, and after intracerebral injection 5-7 months. Only a proportion of injected sheep, however, develop the clinical disease. Wilson, Anderson and Smith (1950) showed that the virus would pass through a gradocol membrane of 41 $m\mu$ A.P.D. Finer filters were not tried. The virus must therefore be less than 16 $m\mu$ in diameter. It is very resistant. It survives drying for at least 2 years if kept in the ice-chest, and is not killed by 0.35 per cent. formalin. There is some reason to believe that the virus may remain latent in rams, which can transmit infection to their offspring. The ewe may remain unaffected, and bear healthy lambs by another ram (see Andrewes 1950).

We append brief descriptions of two animal diseases whose position in our rough scheme of classification is very doubtful.

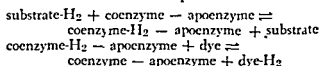
SALIVARY GLAND VIRUS OF GUINEA-PIGS

Cole and Kuttner (1926) observed greatly swollen epithelial cells in the salivary glands of guinea-pigs; they were found in 84 per cent. of full-grown animals. Injection of a suspension of the affected submaxillary glands intracerebrally into young guinea-pigs was followed by fever, cerebral irritation, and death in 5 to 7 days, with diffuse subacute meningitis. In the exudate large numbers of cells were found similar to those in the salivary glands. Similar cells were also seen in the lesions resulting from inoculation of the same suspension into the testicle, lung, tongue, and submaxillary glands of young guinea-pigs. Markham (1933) recorded the occasional finding of characteristic inclusions in the epithelium of the convoluted tubules in the kidney of naturally infected animals. The virus is destroyed at 54° C. in 1 hour; it survives for 11 but not for 28 days in 50 per cent glycerol. It passes through a Berkefeld N candle. The virus appears to give rise to no clinical symptoms in naturally infected guinea-pigs. Infected guinea-pigs are immune to the intracerebral inoculation of virus; in the serum of these animals neutralizing bodies can be demonstrated (Andrewes 1930). More recently Kuttner and Wang (1934) described the presence of acidophilic intranuclear inclusion bodies in the submaxillary glands of hamsters, white mice, and wild rats; each of these animals appears to be infected by a specific virus. Similar inclusion bodies have also been noted in the salivary glands of new-born infants. They are not



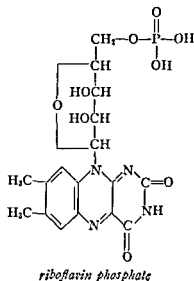
The pyridinoproteins are soluble, relatively easily extracted from cells, and their reduced forms are reoxidized by flavoproteins. In bacteria, the malic, lactic and 3-phosphoglyceraldehyde dehydrogenases are DPN-proteins, while glutamic acid dehydrogenase is a TPN-protein. The *cytochrome-linked dehydrogenases* are associated with the insoluble submicroscopic cell particles. In crude preparations they react with oxygen through the cytochrome system, but there is evidence that an additional hydrogen carrier is involved. It may be a flavoprotein or perhaps cytochrome *b*. The succinic and formic dehydrogenases of *Bact. coli* and the lactic dehydrogenase of the gonococcus are cytochrome-linked.

The oxidation of substrates by dehydrogenases may be conveniently studied in the absence of other respiratory enzymes by means of a technique introduced by Thunberg. In this procedure, the dehydrogenase is reduced by its specific substrate and reoxidized by a reversibly oxidized and reduced dye possessing a suitable oxidation-reduction potential.

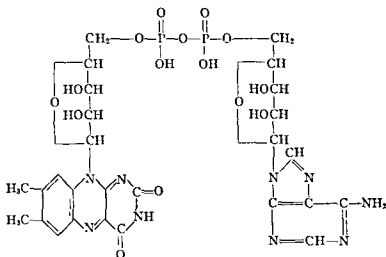


When the oxidized form of the dye is colored and the reduced form is colorless (methylene blue, for example), the rate of such a reaction may be measured in terms of the rate of decolorization of the dye in the absence of oxygen.

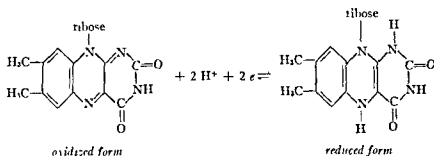
Flavoproteins. The flavoproteins are respiratory enzymes containing either riboflavin phosphate or flavin adenine dinucleotide as coenzymes.



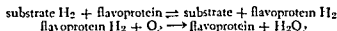
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*flavin adenine dinucleotide*

In both riboflavin coenzymes, oxidation-reduction is confined to the isoalloxazine nucleus.

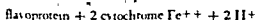
*oxidized form**reduced form*

Derivatives of riboflavin are apparently present in all bacteria and are found in especially large amounts in some anaerobes. Flavoprotein enzymes fall into two functional groups. The *oxidases* catalyze irreversible oxidations of substrates and react directly with oxygen to form hydrogen peroxide.



These flavoprotein oxidases have been studied almost entirely in animal tissues, and no clear-cut demonstration of such an enzyme in bacteria has been made. *Lactobacillus delbrueckii* contains an auto-oxidizable flavoprotein which is probably responsible for the oxygen uptake of this cytochrome-free organism, but the flavoprotein has not been shown to react directly with metabolites. Most peroxide formation in bacteria is probably due to flavoprotein-catalyzed reactions.

Other flavoproteins function in hydrogen transport as the link between the dehydrogenases and the cytochromes.



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These flavoproteins catalyze reversible reactions and do not react with either metabolites or molecular oxygen. Flavoproteins of this type have been studied in *Bact. coli*, and they probably are found in all cytochrome-containing bacteria.

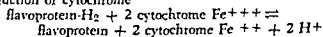
Iron-Porphyrin Protein Enzymes. Several important respiratory enzymes have iron-porphyrin compounds as coenzymes. These iron-porphyrins are closely related to, but not identical with, the heme of hemoglobin. Only the iron atom of the iron-porphyrin undergoes reversible oxidation-reduction. Almost all aerobic cells contain three iron-porphyrin protein pigments, the cytochromes *a*, *b* and *c*. Each of the cytochromes may readily be identified spectroscopically by four sharp absorption bands in the visible spectrum when in the reduced ferrous state, and their presence or absence in a relatively large number of bacteria has been determined.

The cytochromes are almost invariably present in aerobic bacteria and absent in obligate anaerobes, while facultative anaerobes may be lacking one, two or all of the cytochromes. Although some cytochrome-free bacteria, such as *L. delbrueckii*, rapidly consume oxygen, there is no doubt that the cytochromes are intimately associated with sustained aerobic growth and metabolism.

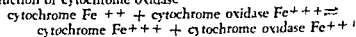
Cytochrome oxidase, Warburg's respiratory enzyme, is a portion of the sub-microscopic particulate structure of the cell, but it may be brought into solution or stable suspension by treatment with ultrasonic vibrations. Although cytochrome oxidase has never been isolated in pure form, its absorption spectrum and its behavior with inhibitors show that it is an iron-porphyrin protein.

The cytochrome system functions in respiration as the last link between substrate and oxygen. The path of the electron and hydrogen transport over the cytochrome system may be divided into three steps:

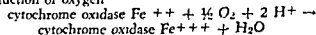
1. Reduction of cytochrome



2. Reduction of cytochrome oxidase



3. Reduction of oxygen

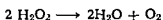


No peroxide can be detected in oxidations catalyzed by the cytochrome system. The mechanism of the reaction between cytochrome oxidase and oxygen (activation of oxygen) is unknown, but Warburg has suggested that oxygen forms a loose combination with cytochrome oxidase similar to oxyhemoglobin; in this complex, the oxygen is activated and accepts electrons and hydrogen to form water. The oxygen consumption of almost all cells is more or less completely inhibited by hydrogen cyanide and carbon monoxide, and the carbon monoxide inhibition is removed by irradiation with visible light. Only the iron-porphyrins combine with both hydrogen cyanide and carbon monoxide, the carbon monoxide compounds being light-dissociable. The importance of the cytochrome system in aerobic respiration is shown by the almost complete inhibition of oxygen uptake by cyanide or carbon monoxide in cytochrome-

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containing cells. It has been found that in respiring yeast cultures each molecule of cytochrome is oxidized and reduced 4000 times a minute, a rate sufficient to account for all the oxygen uptake of the cultures. The portion of bacterial respiration not inhibited by cyanide or carbon monoxide is probably carried out by auto-oxidizable flavoproteins or by other respiratory enzymes with reversibly oxidized and reduced substances such as phthiocol, a yellow pigment of the tubercle bacillus, or pyocyanine, a pigment produced by *Ps. pyocyanea*, as coenzymes.

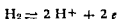
Most organisms contain an enzyme called *catalase* which decomposes hydrogen peroxide.



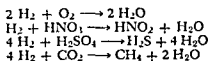
The catalase crystallized from beef liver by Keilin and Hartree is an iron-porphyrin protein and it is generally accepted that catalase from other sources has a similar chemical nature. Catalase is present in almost all aerobic bacteria and some facultative anaerobes, but under usual conditions of culture it cannot be detected in *Clostridium* and some of the streptococci, lactic acid bacteria and dysentery bacteria.

The *peroxidases* are enzymes, probably iron-porphyrin proteins, which catalyze the oxidation of substrates by hydrogen peroxide. Their distribution in bacteria parallels that of the cytochromes and catalase, and their function in bacterial metabolism is unknown.

*Hydrogenase*¹⁰ is a bacterial enzyme which catalyzes the reaction of the normal hydrogen electrode



In the presence of hydrogenase and molecular hydrogen, many substrates are reduced.



Hydrogenase activity was first found in the autotrophic hydrogen bacteria and much later in many heterotrophic facultative anaerobes such as *Bact. coli*. Recently, hydrogenase has been observed in the strongly aerobic *Azotobacter* where it is apparently involved in some manner with nitrogen fixation. Although hydrogenase has never been isolated, it is probably an iron-porphyrin protein because it is inhibited by hydrogen cyanide and by carbon monoxide, the latter inhibition being light-reversible, and because it is not found in cultures grown on iron-deficient mediums. Hydrogenase is inactivated by molecular oxygen, and it has been suggested that the Fe^{++} form is active while the Fe^{+++} form is inactive.

The Relation of Bacteria to Molecular Oxygen. The bacteria differ from one another in their relationship to molecular oxygen. Certain bacteria, the *obligate aerobes*, require ready access to air, growing feebly or not at all

¹⁰ Recent investigations on hydrogenase are discussed in considerable detail by Lipmann: *Ann. Rev. Biochem.*, 1943, 12:5, and Stephenson: *Antonie van Leeuwenhoek*, 1947, 12:33.

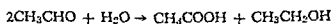
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in its absence. Such organisms may possibly lack certain respiratory enzymes necessary for anaerobic respiration, but there is no proof of this. It is also possible that end products of their anaerobic metabolism are toxic to them, or that they cannot grow in the presence of strong reducing intensities. This group includes such well known bacteria as the nitrifying bacteria, some of the sulfur bacteria, *Bacillus subtilis* and related forms, *Azotobacter*, the diphtheria bacillus, the cholera vibrio, and others.

However, most bacteria can grow in the complete or virtual absence of molecular oxygen. Two types of anaerobic bacteria may be distinguished. The most numerous are the *facultative anaerobes*, which respire with equal facility in the presence or absence of molecular oxygen. In broth cultures, the dissolved oxygen is soon exhausted and, after a preliminary period of aerobic growth, the culture continues to develop under essentially anaerobic conditions. The *obligate anaerobes* are unable to grow in the presence of molecular oxygen, and oxygen is actively toxic to them; vegetative cells die very quickly upon exposure to air, although spores are highly resistant. Members of the genus *Clostridium*, together with microaerophilic forms which tolerate very low concentrations of oxygen, are usually included in this group.

Bacteria are unexcelled in the possession of powerful and varied means for obtaining energy from nutrients in the absence of oxygen. The energy obtained from a given quantity of nutrients is usually much less under anaerobic conditions than in the presence of oxygen, because oxidation of substrates is less complete, and anaerobic metabolism is characterized by a rapid utilization of oxidizable substrates and a great accumulation of partially oxidized end products.

Bacteria carry out energy-yielding oxidations under anaerobic conditions in a number of ways. Inorganic compounds may be reduced and thus replace molecular oxygen as the final electron acceptor. For example, ammonia and a single organic compound such as lactic acid are sufficient to support the aerobic growth of *Bact. coli* in a medium of inorganic salts, but growth in the absence of air does not occur unless nitrate ion is also present and the nitrate reduced to nitrite by the enzyme nitratase. Organic compounds may also act as final oxidants in anaerobic respiration. Thus the reduction of fumaric acid to succinic acid by succinic dehydrogenase may replace the reduction of nitrate in supporting the anaerobic growth of *Bact. coli*. Another energy-yielding reaction is a reaction between two substrates in which one is reduced and one is oxidized. This type of reaction is called a *dismutation*, and is a very common type of anaerobic reaction in bacteria. For instance, acetaldehyde may dismutate to acetic acid and ethanol.



While carbohydrate is the chief energy source for both aerobic and anaerobic heterotrophes, certain of the obligate anaerobes possess only a very limited ability to metabolize carbohydrate, and an important source of energy for these bacteria appears to be the coupled oxidation-reduction of pairs of amino acids. *Cl. sporogenes*, for example, carries out the reaction between alanine and proline at a rapid rate¹¹ (see accompanying equation).

¹¹ Suckland *Biochem. J.*, 1934, 28:1746, 1935, 29:889.

CHAPTER 88

FILTRABLE VIRUS DISEASES—*Continued*

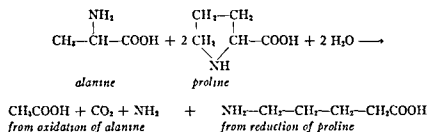
D. GROUP CHARACTERIZED BY CATARRHAL OR GENERALIZED INFECTION

MEASLES

MEASLES is a highly infectious disease of human beings, which is endemic throughout the world. In Europe and America epidemics tend to occur in alternate years, as a fresh susceptible population of children grows up. Among civilized peoples it is chiefly a disease of childhood, fairly mild in itself, but when complicated by respiratory infections—as it so often is—is apt to be attended by a high case-fatality rate. Introduced, however, into non-endemic areas it affects young and old alike, and takes on a more malignant character. When it was imported, for example, into the Fiji islands in 1875, it carried off 20-25 per cent. of the entire population (see Brueker 1938). Most children exposed to infection for the first time contract the disease. For instance, in the epidemic in southern Greenland described by Christensen and his colleagues (1953) the attack rate was 999 per 1,000. There is some evidence, however, that during epidemics a small proportion of children may develop a latent infection resulting in a temporary or sometimes permanent immunity (see Halliday 1928, Stocks and Karn 1928, Enders 1941).

In this country measles is a disease of winter and spring, the maximum mortality usually being recorded in April. The number of deaths is now far less than it used to be—partly perhaps because of the better nutritional state of the children and partly because of the introduction of prophylactic serum and of the antibiotic treatment of secondary infections. The incubation period is usually 10-11 days, depending on whether the fever or the rash is counted as the beginning of the disease. Infectiousness appears to be greatest during the catarrhal stage, but lasts from about 4 days before to 5 days after the appearance of the rash. Comparing measles, varicella, and mumps, Simpson (1952) found that the age distribution of these diseases was inversely related to their degree of infectiousness, measles attacking the younger and mumps the older children with varicella coming in between.

Anderson and Goldberger (1911) first recorded evidence indicating that measles could be experimentally transmitted to monkeys. The experiments of Nicolle and Conseil (1911, 1920) likewise suggested that monkeys were susceptible. The most convincing evidence, however, was brought by Blake and Trask (1921). These workers were able, by the intratracheal inoculation of filtered and unfiltered nasopharyngeal washings taken from measles patients 6 days before to 22 hours after the appearance of the rash, to produce in monkeys (*Macacus rhesus*) a disease closely simulating human measles. After an incubation period of 6 to 10 days, there developed a characteristic group of symptoms



As already mentioned, the obligate anaerobes contain none or very little of the iron-porphyrin respiratory enzymes, a definite indication of enzymatic deficiency. However, the toxicity of molecular oxygen to these bacteria requires more explanation than their lack of oxygen-transporting enzymes. The catalase theory is based on the discovery that certain bacteria produce hydrogen peroxide in amounts sufficient to be toxic to themselves.¹² In cultures of those species which do not produce catalase and are peroxide-sensitive, peroxide accumulates and the culture becomes self-sterilizing; the pneumococcus is an example of this type of microorganisms. Those bacteria which produce catalase decompose the peroxide as it forms and it does not accumulate to toxic concentrations. The obligate anaerobes are peroxide-sensitive and do not form catalase. Hence it has been suggested that these organisms form peroxide rapidly in the presence of air and therefore oxygen is toxic to them. Although this theory is accepted by some, it entirely lacks supporting evidence, for all attempts to demonstrate peroxide in cultures exposed to air have failed.

It has also been proposed that the reducing intensity of the medium is the factor governing whether or not growth will occur. Experimental evidence, both direct and indirect, has been presented which indicates that a certain degree of reducing intensity is essential to the germination of tetanus spores¹³, the positive limit appears to be about +50 millivolts at pH 7.0. It has been suggested that this accounts for the failure of tetanus spores to germinate in healthy tissues in the absence of trauma or secondary infection as is sometimes observed. Since a sufficient reducing intensity cannot be maintained in the presence of oxygen, oxygen is toxic. It is not clear why such a reducing intensity should be required. Possibly some of the respiratory enzymes of the obligate anaerobes are oxygen-labile, i.e., active in the reduced state and inactive in the oxidized state, as are some of the bacterial hemolysins (p. 207) or hydrogenase (p. 73), or perhaps the substrates of some of the enzymes of these organisms are unstable under aerobic conditions.

The Role of Phosphorylated Compounds in the Conservation and Utilization of the Energy Liberated in Respiration.¹⁴ Phosphorylated organic compounds are of primary importance in the energy metabolism of bacteria and all other living things. Phosphorylated compounds of biological importance may be conveniently divided into two groups according to the energy liberated upon hydrolysis of the linkage involving the phosphorus.

¹² McLeod and Govenlock. *Lancet*, 1921, 1900.

¹³ Knight and Fildes. *Biochem. J.*, 1930, 24 1496. Quastel and Stephenson. *Biochem. J.*, 1925, 20 1125.

¹⁴ Comprehensive discussions of this subject have been given by Lipmann. *Advances in Enzymology*, 1941, 1 99, and Kalckar. *Chem. Rev.*, 1941, 28 71.

healthy home contacts from attending school, provided that they are kept under observation. In view of the high complication rate due to cross-infection with hæmolytic streptococci, treatment at home rather than in hospital is advocated for patients with uncomplicated measles (see Wright 1915).

The specific prophylaxis of measles is greatly aided by the use of human serum or its derivatives. The new-born infant, so long as its mother has had measles, is passively protected for the first 4-6 months of life. From then up till 12 months of age it is advisable to prevent an attack completely. After that the aim of serum prophylaxis should be to produce an attenuated attack. However, in weakly debilitated children, in children suffering from another disease, and sometimes in hospitals and institutions, complete protection should be aimed at. When complete protection is attained, the duration of the resulting immunity is 3-4 weeks, and the patient is subsequently susceptible to infection. The immunity following an attenuated attack appears to be permanent.

The various products that may be used are : (a) *Whole blood*. This is of value mainly in general practice when blood may be withdrawn from the mother or other member of the household and injected directly into the child. The objection to whole blood is that it may sensitize the recipient to rhesus agglutinin : it should therefore be avoided, if possible, in female children (Marshall 1918). (b) *Adult serum*. This is satisfactory if the blood is withdrawn from normal adults who have previously suffered from measles, but the requisite dosage is fairly high (see Brincker 1936). (c) *Convalescent serum*. Serum prepared from the blood of convalescent patients taken 7-10 days after defervescence has about twice the protective power of normal adult serum and hence can be given in half the dosage. (d) *Placental extract*. This was recommended by McKhann and Chu (1933) and McKhann (1937). In potency it occupies a place midway between adult and convalescent serum. The difficulty, however, of extracting the placental globulins and the high proportion of local and systemic reactions that follow its use have precluded it from general acceptance. (e) *Gamma globulin*. This can be extracted from human plasma either by the ethanol-water method of protein fractionation devised by Cohn and his colleagues (1946) or by the ether method of Kekwick and Mackay (1951). Both products contain the specific antibody in greater concentration than convalescent serum, cause only few and mild reactions, and prove on the whole very satisfactory in practice (Cohn *et al* 1944, Stokes *et al* 1944, Ordman *et al* 1944, Greenberg *et al* 1944, Report 1950).

Which of these preparations should be selected must depend on circumstances. In the past adult serum has been extensively used, with a considerable measure of success. The occasional occurrence, however, of serum hepatitis after its use, sometimes with serious and even fatal effects (see p 2207), has brought it into gradually increasing disfavour. Attempts to destroy the hepatitis virus in the serum by ultraviolet irradiation have proved disappointing, and the tendency now is to replace serum as completely as possible by gamma globulin, which, so far as present experience goes, appears to be free from the risk of causing this particular complication.

When gamma globulin is not available and adult serum has to be used, it should be injected intramuscularly, preferably into the vastus externus. Complete protection is difficult to secure, unless large doses are given within 5 days of exposure to infection. For attenuation, the dose may be estimated, in millilitres, as the age of the child multiplied by 2 when the injection is made within 5 days of exposure,

of rubella proved refractory to experimental infection, suggesting that they had previously suffered from the disease in a latent or sub-clinical form. Krugman and his colleagues (1953) were likewise successful in transmitting the disease experimentally to human volunteers by intramuscular injection of blood serum and by nasal and oral administration of nasopharyngeal washings. In their experience the incubation period was only 9-16 days. The virus can be preserved at -70°C . for at least 9 months. Anderson (1954) reported its cultivation in roller tube tissue cultures of adult monkey kidney. (See also Habel 1942.)

Gregg (1941) and Swan and his colleagues (1943, 1944) in Australia drew attention to the prevalence of abnormalities in infants born of mothers who had suffered from rubella during pregnancy. Congenital cataract, deaf-mutism and cardiac disease are the commonest defects, but numerous others such as strabismus, cleft palate, spina bifida, and microcephaly have been reported. There is reason also to believe that pregnancy may end in stillbirth (Swan 1948). Complications are most likely if rubella occurs in the first 3 or 4 months of pregnancy, the 2nd and 3rd being apparently the most dangerous (Swan and Tostevin 1946). These observations have been confirmed in many other countries (see Ober *et al* 1947, Clayton-Jones 1947). How frequently maternal rubella in pregnancy results in abnormalities of the infant, it is difficult to say; the few figures available are rather conflicting. Rubella is probably not the only virus disease to be transmitted to the foetus. Aycock and Ingalls (1946) bring evidence to suggest that poliomyelitis during pregnancy may lead to congenital abnormalities; and there is other evidence that measles and chicken-pox may occasionally act in the same way.

In view of these findings it is highly desirable, for girls at any rate, to have rubella before puberty. Whether it is justifiable to expose them deliberately to infection or to transmit the disease to them experimentally, is open to discussion, but so long as the subjects are healthy and there is no fear of any other infection being transmitted simultaneously there is a good deal to be said for it. It should be remembered that the disease produced experimentally is contagious and that it may not be accompanied by a rash. Care should therefore be taken to prevent the infection spreading to pregnant women who have not had the disease. Pregnant women without a history of rubella who are exposed to infection should be given gamma globulin, preferably made from convalescent serum, as soon as possible in the hope that it may forestall the disease, but so far the evidence of its value in this respect is unconvincing (see Korns 1952, Anderson and McLorinan 1953).

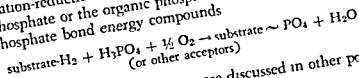
MUMPS

Mumps is an infectious disease of human beings characterized by a non-suppurative enlargement of the parotid glands, and sometimes of the testicles. It is commonest between 5 and 15 years of age, but often attacks young adult recruits to the fighting services. Most cases occur in the spring. The incubation period is variable; it is usually given as 12-26 days with 18 days as the commonest. Infectivity probably lasts from a day or two before symptoms appear to the disappearance of the glandular swelling. Transmission occurs directly or indirectly from infected saliva. The infectiousness is much lower than that of measles or chicken-pox, and latent and sub-clinical attacks are frequent (Maris *et al* 1946, Simpson 1952). The disease seldom proves fatal, and even the meningitis and encephalitis that may follow it are usually benign. Second attacks are uncommon.

The Role of Phosphorylated Compounds

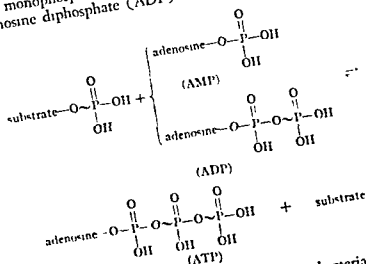
two compounds. The differences in chemical behavior between compounds with low energy and high energy phosphate bonds are similar to those between ethyl acetate, an ester of acetic acid and ethanol, comparable to low energy phosphorus compounds, and acetyl chloride, an anhydride of acetic acid and hydrochloric acid, comparable to high energy phosphorus compounds. When water is added to ethyl acetate, no heat (energy) is evolved and the ester is not hydrolyzed. If water is added to acetyl chloride, heat is evolved with explosive violence and acetyl chloride is quickly split into its two components. Because of the differences in energy content, compounds with low energy phosphate bonds can never be in reversible equilibrium with compounds containing high energy bonds. Compounds with phosphate bonds of the same energy content may be in equilibrium with each other, and high energy phosphate bonds may be used to form low energy phosphate bonds.

In both the autotrophic and heterotrophic bacteria, the energy released in the oxidation-reduction reactions of respiration may be used to convert inorganic phosphate or the organic phosphate of low bond energy compounds into high phosphate bond energy compounds



Specific examples of this general process are discussed in other portions of the chapter (*vide infra*).

The adenine nucleotides are of particular importance in the transfer of high energy phosphate bonds from place of generation to place of utilization. The high energy phosphate bonds generated in respiration are transferred to adenosine monophosphate (AMP, adenylic acid) or, as is more often the case, to adenosine diphosphate (ADP) and conserved in the form of ATP.



The energy-rich phosphate bonds of ATP are used by bacteria in the assimilation of carbon dioxide, the phosphorylation of carbohydrates and the synthesis of compound sugars (*vide infra*). More generally, it has been demonstrated that ATP is involved in muscular contraction, the synthesis of peptide bonds, the oxidation of fatty acids and many other biochemical processes.

in the infected chick embryo. One, the V antigen, is closely linked with the virus in the amniotic and allantoic fluids: the other, the soluble or S antigen, is smaller in size and is present chiefly in the membranes (Henle, Harris and Henle 1948). In mumps, antibodies to the S antigen appear early in the disease and reach high levels before antibodies to the V antigen begin to rise. During early convalescence both antibodies are demonstrable in high titre. Later, the S antibodies usually decline more quickly than the V, so that after some years only V antibodies may be found. S antibodies may be detectable on the 1st or 2nd day of the disease, and are sometimes of value in the diagnosis of meningo-encephalitis in the absence of glandular swelling. (c) *Hæmagglutination-inhibition test* The patient's serum is examined for its ability to prevent agglutination of chicken red corpuscles by the virus. The usual two specimens of sera, taken during the acute stage of the disease and during convalescence respectively, are required to show a rise in antibody titre. The test is said to be inferior in value for diagnostic purposes to the complement-fixation test (Lundback 1949). (d) *Modified human red cell agglutination test*. Burnet (1946) found that human red blood corpuscles treated under certain conditions with mumps virus were specifically agglutinated by mumps antibody. This test is very sensitive and the end-point may be difficult to determine (Aikawa and Meiklejohn 1949). (e) *Serum neutralization test*. Gotlieb and his colleagues (1953) developed a neutralization test for mumps in the chick embryo using serial dilutions of inactivated serum and constant amounts of virus. Convalescent sera showed a wide range of neutralizing activity from 1/8 to over 1/2,000. The neutralizing titres did not run parallel to those obtained by the complement-fixation and hæmagglutination-inhibition tests, which themselves yielded different results. (f) *Skin test*. When a suspension of heated inactivated mumps virus from the parotid glands of infected rhesus monkeys is injected intradermally into a person who has had mumps, an erythematous reaction occurs reaching 10–15 mm. or more in diameter within 48 hours (Enders *et al.* 1946). As an index of immunity the test appears to be of much the same value as the complement-fixation test (Henle *et al.* 1951). (For serological confusion with the Newcastle virus, see p. 2230.)

Protection.—Attempts to produce active immunity against mumps are still in the experimental stage. Virus grown in eggs may be inactivated by ether, formalin, or ultraviolet irradiation, or a living attenuated virus may be used. None of these vaccines, however, has so far proved capable of giving rise to a firm persistent immunity (see Stokes *et al.* 1946, Habel 1951, Bashe *et al.* 1953). It should be added that mumps is not usually a serious disease, and vaccination should seldom be called for except in special circumstances, such perhaps as the protection of hospital staffs and of groups of young adults undergoing military training. Babies born of mothers that have had mumps are immune by virtue of passively transferred antibody. Some degree of passive protection may perhaps be conferred by the injection of convalescent serum, or better gamma globulin, within five days of exposure to infection. The gamma globulin should be prepared from convalescent serum and should be given in a dose of about 20 ml. to an adult. Its chief virtue is to lower the risk of developing orchitis, which so often complicates the disease in adult life (see Gellis *et al.* 1945b).

THE AUTOTROPHIC BACTERIA

Of the many types of physiological activity occurring among the bacteria, that of the so-called *autotrophes* is unique in biochemical physiology. The respiratory mechanisms of chemosynthetic organisms are concerned, for the most part, with the oxidation of organic molecules. Such organisms are generally spoken of as *heterotrophes*, organisms which are dependent upon the organic substances synthesized by other living cells. The autotrophic bacteria, however, derive the energy necessary to their life processes from the oxidation of inorganic compounds of nitrogen, sulfur and iron, and obtain nitrogen from ammonium salts and carbon from carbonate or carbon dioxide.

The Nitrifying Bacteria. Of the autotrophic bacteria perhaps the most important single group is that of the microorganisms, discovered by Winogradsky in 1890, which oxidize ammonia to nitrite and nitrite to nitrate. The species of bacteria concerned are *Nitrosomonas* and *Nitrosococcus* (so-called because their cultures give the nitroso reaction in the qualitative test for nitrite) which bring about the reaction:



and *Nitrobacter* which brings about the reaction:



These equations represent the reactions from which these organisms obtain energy for maintenance and growth. In the presence of ammonia and nitrate organic compounds are not oxidized, and their presence may even inhibit growth.¹⁶ However, Boltjes has observed a favorable influence of certain protein derivatives and fatty acids upon the growth of nitrifying bacteria, and Bomeke has shown that, in the absence of ammonia and nitrite, these organisms carry out an oxidation of organic substances, presumably of endogenous origin.

The relative simplicity of the respiratory mechanisms of these two species of nitrifying bacteria has made possible a reliable determination of the free energy efficiency of these organisms. The ratio of nitrogen oxidized to carbon fixed as protoplasm has been found to be 35 for *Nitrosomonas* and 135 for *Nitrobacter*. Assuming the energy consumed in the synthesis of protoplasm to approximate that of the reduction of carbon dioxide to hexose, the efficiency of *Nitrobacter* becomes 7.8 per cent and that of *Nitrosomonas* 5.9 per cent.¹⁷ Presumably, then, about 95 per cent of the energy liberated should appear as heat, and experiment has shown this to be true. As machines, therefore, the nitrifying bacteria are not highly efficient.

The actual process of oxidation is entirely unknown; it has been suggested that hydroxylamine and hyponitrous acid may be intermediates, but there is no evidence for this.

¹⁶ For example, 0.015 M glucose completely inhibits *Nitrobacter* in liquid media and somewhat higher concentrations, 0.2 per cent, are necessary to inhibit growth in sand media or soil. (Coleman: *Centralbl. f. Bakt.*, 1908, Abt. II, 20:401, 485.)

¹⁷ Calculated on a free energy rather than heat of combustion basis. Cf. Bass Becking and Parks: *Energy Relations in the Metabolism of Autotrophic Bacteria*. *Physiol. Rev.* 1927, 7:85.

Robertson and Felix (1930) found that an antiserum to *Cl. septicum*, containing antibodies against the heat-stable somatic antigen, but none against the heat-labile flagellar antigens, had a high protective value in mice. Henderson (1932) recorded analogous results with *Cl. chauvæi*. These results accord in general with those found in the salmonellæ. But later Henderson (1937) observed that antisera to unheated formalized *Cl. septicum* contained a protective antibody specific for a heat-labile antigen in the bacilli. The antigen had none of the qualities of the heat-labile Vi antigens, and appeared to be flagellar in nature, the protective effect being roughly proportional to the flagellar agglutination titre. Henderson suggested that flagellar antibody might exert an immobilizing effect on a motile, pathogenic bacterium and thus prevent its dissemination through the tissues.

The Efficacy of Antibacterial Immunity.

As we have already emphasized in Chapter 42, immunity may be of very varying grade. The first effect of the mechanisms that we have discussed in the present chapter is to free the circulating blood from bacteria, and so save the host from a fatal bacteræmia. The effect on the local foci of infection is less pronounced, and much less rapid. Bacteria may remain latent in such foci over considerable periods.

Thus (Topley 1929) of 64 vaccinated mice that had survived in apparent health for 28 days after an intraperitoneal injection of 1,000 virulent *Salm. typhi-murium*, 31 were found to be harbouring that organism in the spleen. We need not doubt that a complete sterilization of the tissues often occurs; but we must not identify the absence of overt illness with a complete absence of infection, or assume that illness followed by recovery is identical with a return to the uninfected state.

There is another factor which may limit the efficacy of immunity of this type. There are regions of the body in which bacterial infections are particularly dangerous, and when foci are established at these sites an immunity that is effective against lesions elsewhere seems to be of little avail. Thus Bull (1915*b*) noted that immunized animals, after rapidly freeing their blood stream of pneumococci and remaining in apparent health for several days, might later succumb to a pneumococcal meningitis. The particular susceptibility of certain regions of the body, even in the immune animal, may be an inherent feature of the animal. It may also be a reflection of variations in the distribution and efficacy of antibody in the various tissues. We shall examine this latter possibility in the next section.

In general, antibacterial immunity is, as we should expect, more variable and often less effective than antitoxic immunity

The Conditions under which Antibodies exert their Protective Action.

The union of antibody with the surface antigens of an invading bacterium clearly renders the parasite more susceptible to the defence mechanisms of the infected host—for example, to the lethal and bacteriolytic action of complement and to phagocytosis. The importance for protection of the host of phenomena like precipitation and agglutination is less well established. The formation of visible precipitates, or of an agglutinated mass of bacteria, in the tissues or tissue fluids of an immune animal cannot take place unless there is an adequate concentration of antigen, either in the form of soluble products of bacteria, or of bacteria themselves.

In discussing the functional significance of precipitins Cannon (1940) suggests that specific aggregates in the body range in size from molecular aggregates up to large precipitates. The *in vivo* formation of large precipitates is, however, mostly a matter of

The Sulfur Bacteria

The organisms may be isolated by the inoculation of mineral salt solutions with soil, the nutriment being carbonate and an ammonium salt in the case of *Nitrosomonas* and *Nitrosococcus* and carbonate and nitrite for *Nitrobacter*. Isolation by the usual pour plate pure culture methods is difficult if not impossible, but colonies may be secured by inoculating silica gel plates from the enrichment cultures. *Nitrosomonas* grows in microscopic, colorless colonies which turn brown on continued incubation, while *Nitrobacter* produces somewhat larger colonies, light brown in color.

The oxidation of ammonia is brought about not by a single species of bacterium but rather by a group of closely related organisms. *Nitrosomonas europaea*, found in western Europe, is a coccobacillus about 1.5 by 1.0 μ and exists in two forms, one a zooglea of closely packed cells and the other an actively motile swarming (monas) stage in which each cell has a single flagellum. Morphologically similar organisms have been found in this country and elsewhere, although in some cases one stage appears to predominate over the other to such a degree that some might be taken to be different organisms. Coccus forms, given the generic name *Nitrosococcus*, have also been isolated in various parts of the world. A number of species have been reported which some workers group under the single head of *Nitrosococcus nitrosus*. These organisms are all gram-positive, obligate aerobes. The nitrite oxidizing organisms appear to be somewhat more homogeneous. All are rod-shaped, 1.0 by 0.3 to 0.4 μ in size, with one or both ends pointed, non-motile and gram-negative or gram positive. Neither *Nitrobacter* nor the ammonia oxidizing organisms form spores.

The Sulfur Bacteria. The group of autotrophic bacteria that derive their energy from the oxidation of sulfur and its compounds is, both morphologically and physiologically, somewhat more complex than that comprising the nitrifying organisms. Some of the organisms, the filamentous forms, are more closely related to the lower fungi than are the so-called "true bacteria." *Beggiatoa*, one of the common forms, shows a close resemblance to the blue-green alga *Oscillaria*. Others, such as *Thiobacillus*, are morphologically indistinguishable from the usual bacteria. In all, three morphological groups may be observed:

- (1) The thread forming, filamentous forms usually regarded as "higher bacteria" which accumulate sulfur granules within the cells. Three genera are ordinarily included in this group: *Beggiatoa*, *Thiothrix* and *Thioploa*.
- (2) Non thread forming organisms existing in a number of forms and including such genera as *Thiobacillus*, *Thiospirillum*, *Thiovulum*, *Achromatium*, etc. *Thiobacillus* is often separated from the other organisms on the basis of the failure to accumulate sulfur granules within the cells.
- (3) The purple and green sulfur bacteria, which differ from the other two groups in that they are pigmented, the pigments making possible a photosynthetic metabolism.

The sulfur bacteria are widely distributed over the earth and are commonly found in the water of sulfur springs, in sewage-laden streams, in swamps where masses of vegetable matter are undergoing slow decomposition and, in fact, wherever sulfur and hydrogen sulfide are present. Whether such organisms bear any relation to the laying down of sulfur deposits is uncertain. It has been suggested that possibly gypsum deposits may have

Bennett and Stokes (1952) of an outbreak in an orphanage lasting over a period of 8 years supports the view that close contact with faecal material is the common mode of infection. The clinically manifest disease was almost completely confined to a group of student nurses. These became infected from the infants and children among whom there was a continual series of anicteric cases. The institution of a nursing technique designed to reduce the risk of infection from the faeces brought the outbreak in the nurses to an end, even though no steps were taken to control infection by the respiratory tract. The pathogenesis of the disease is by no means clear, and it may be that some activating factor, such as that met with in infectious hepatitis of mice (see p. 2211), is necessary to enable the hepatitis virus to produce its full effect.

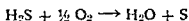
Though various animals, such as pigs, mice, rats, and canaries, are said to have been infected experimentally (see Andersen and Tulinius 1938, Dresel and Weineck 1950, Wildfuhr 1951), no satisfactorily reproducible results have been obtained by this means. Most of our knowledge about the infective agent has been derived from transmission experiments on human beings. These have shown that volunteers can be infected with faeces or blood taken during the first few days of the disease, but not with pharyngeal secretions or with urine (Voegt 1942, Cameron 1943, MacCallum and Bradley 1944, Neefe and Stokes 1945, Havens 1946a, Report 1951b). An exception may perhaps be made for urine, which Findlay and Willcox (1945) found to be infective in natives suffering from bilharziasis. The agent, which is sometimes referred to as Virus A, can pass through a Seitz filter, and proves infective by subcutaneous injection as well as by the mouth (Cameron 1943, Neefe and Stokes 1945, Findlay and Willcox 1945). The incubation period after oral infection is the same as in the natural disease, namely 15-40 days.

Early attempts to cultivate the virus proved a failure; but W. Henle and his colleagues (1950) reported success by the inoculation of serum and of filtered stools from cases during the acute stage into tissue cultures of rabbit liver cells in roller tubes and minced chick embryos in Simms-Sanders medium followed by passage through the amniotic cavity of the chick. Strains were isolated from two separate outbreaks, and reproduced an anicteric form of the disease in human volunteers infected by the mouth after an average incubation period of 24 days (Drake *et al.* 1950). Confirmation of these results is still awaited.

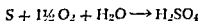
Apart from exclusion of a known pathogenic agent and of Weil's disease, bacteriology can be of little help in the diagnosis of infective jaundice. Though there may be a transient leucocytosis in the early stage of the disease, leucopenia is the rule. There is a rise in the relative proportion of monocytes, but less than that in glandular fever. The blood picture alone is often of value in the differential diagnosis from Weil's disease, in which there is a polymorphonuclear leucocytosis. No specific complement-fixation test has yet been worked out (Miles 1946). G. Henle and her colleagues (1950) described a skin test with an antigen prepared by irradiation of infected amniotic fluid. The test is positive in about half the cases during the course of the disease, and in 90-100 per cent. of persons who have recovered from it. Its value therefore appears to lie more in indicating the existence of immunity to the disease than in diagnosis of an actual attack (Bennett *et al.* 1952). The virus resists heating at 55° C for over 1 hour, but can be destroyed under certain conditions by ultraviolet irradiation. In water it survives chlorination with 1 p.p.m. of chlorine for over 30 minutes, but is attenuated by 15 p.p.m. (Neefe *et al.* 1945).

resulted from the microbial oxidation of sulfur to sulfate neutralized by calcium salts present in the earth.

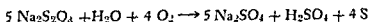
The Sulfide Bacteria. The oxidation of sulfur compounds by these organisms proceeds through a series of stages. The so-called "sulfide bacteria," including the filamentous forms, derive their energy primarily through the oxidation of hydrogen sulfide to elementary sulfur, which is deposited as granules within the cells:



The reaction is the energy-yielding process of these organisms. If the supply of hydrogen sulfide runs low, the organisms further oxidize the accumulated sulfur to sulfate.

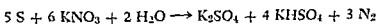


The Thionic Acid Bacteria. A second physiological group, called the thiosulfate or thionic acid bacteria, obtains its energy primarily through the oxidation of thiosulfate, though some members are capable of oxidizing elementary sulfur. The obligate autotrophs, *Thiobacillus thioparus*, is included in this group, together with *Thiobacillus denitrificans*. Some strains of the latter have been found to be obligate autotrophs and also obligate anaerobes, growing in the presence of nitrate, while other strains are both facultative autotrophs and facultative anaerobes. *Thiobacillus thioparus* accumulates free sulfur outside of the bacterial cells when grown in thiosulfate media and the oxidation is supposed to proceed thus:

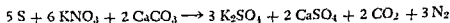


This organism is also able to oxidize sulfide and tetrathionate but oxidizes elementary sulfur to sulfate only very slowly. A number of common enteric bacilli, however, are able to reduce tetrathionate.

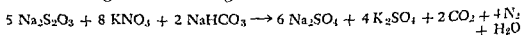
The oxidation of sulfur and reduction of nitrate by *Thiobacillus denitrificans* growing under anaerobic conditions proceeds either as:



or, in the presence of calcium carbonate, as.



The organism is able to oxidize thiosulfate anaerobically in the presence of nitrate according to the following reaction.



The Sulfur Bacteria. A third group, the sulfur bacteria, is represented by only one species, *Thiobacillus thiooxidans*, which derives its energy primarily through the oxidation of elementary sulfur to sulfate. It is also able to oxidize thiosulfate to sulfate, both oxidations proceeding as indicated above. This strictly autotrophic organism is remarkable for its tolerance of high acidities. Sulfuric acid accumulates in its cultures as a result of the oxidation of sulfur, and normal growth takes place in concentrations as high as 0.25 molar, and even 0.5 molar does not completely inhibit growth. Although a strict auto-

(Lehane *et al.* 1949). Calculations indicated that at least 0.35 per cent. of donors must have been carrying the icterogenic virus.

The incubation period of serum hepatitis is usually 2-5 months. The morbidity is often high. In some outbreaks the case-fatality rate has been negligible; in others it has been considerable. There is at present little evidence to suggest that serum jaundice is contagious. Experiments on human volunteers have shown that the serum of patients suffering from the disease can give rise to jaundice on subcutaneous injection, and that the infective agent is present in the blood during the latter part of the incubation period, during the acute stage, but not usually during convalescence (Oliphant *et al.* 1943, Havens 1946*b*), though it has been demonstrated in the blood for as long as 5 years after an attack (Report 1953*a*). The virus, which is sometimes known as Virus B, does not appear to be present in the stools, nor can infection be transmitted by the oral route. Filtration experiments show that it can pass through a gradocol membrane of A.P.D. 52 μ , suggesting that it is probably not more than 26 μ in diameter (see Report 1953*a*).

Relationship of serum hepatitis to infectious hepatitis.—Opinion is divided on the relationship between these two diseases. The general symptomatology, the changes in the white cells of the blood, and the primary necrosis of the liver are all compatible with the view that they are caused by the same infective agent (see Dible *et al.* 1943, Sheehan 1944). Moreover, the causative agent of both diseases is fairly heat resistant, withstanding a temperature of 56° C. for at least 1 hour. On the other hand, there are several differences between them, some of which are dependent on the different methods of transmission (Table 179).

TABLE 179

DIFFERENCES BETWEEN INFECTIOUS HEPATITIS AND SERUM HEPATITIS.

Infectious Hepatitis	Serum Hepatitis
1. Contagious	Apparently not contagious
2. Incubation period 15-40 days	Incubation period 60-160 days
3. Onset acute	Onset insidious
4. Febrile course	Little or no fever
5. Affects children and young adults	Affects all ages
6. Commonest in autumn and winter	Occurs all the year round
7. Virus present in stools	Virus apparently not present in stools
8. Disease experimentally transmissible by mouth	Disease not experimentally transmissible by mouth
9. One attack confers some immunity to infectious hepatitis but not to serum hepatitis	One attack confers some immunity to serum hepatitis, but not to infectious hepatitis
10. Skin test with hepatitis virus positive . .	Skin test with hepatitis virus negative

It may be added that an attack of serum hepatitis not only fails to confer immunity against infectious hepatitis but actually seems to predispose to it (Gauld 1947). None of these differences by itself is conclusive evidence that the two viruses are distinct. The longer incubation period of serum hepatitis, for example, may result from partial neutralization of the virus by antibodies in the serum of the donor or recipient (Aycock and Oren 1947). The failure of cross-immunity may be ascribable to differences in antigenic constitution, and the non-pathogenicity of the serum hepatitis virus by the mouth to differences in virulence. The balance of opinion perhaps favours the dualistic conception (Report 1953*a*); but this leaves us with the problem of deciding how serum jaundice is normally propagated in the absence of parenteral injection. Since, moreover, it is known that parenteral

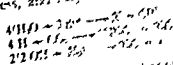
The Sulfur Bacteria

trophe, *Thiobacillus thiooxidans* is not so sensitive to the presence of organic matter as are some other autotrophes. The work of Vogler and of Umbreit has clarified the mechanism whereby the energy released in the oxidation of sulfur to sulfate is used for the assimilation of carbon dioxide into organic compounds. The oxidation of sulfur in the absence of carbon dioxide is accompanied by the uptake of inorganic phosphate and the formation of ATP. If then carbon dioxide is supplied to the bacteria, even in the absence of oxygen, carbon dioxide is rapidly assimilated and inorganic phosphate is liberated. It thus appears that the energy released in the oxidation of sulfur is stored in the energy-rich bonds of ATP, in which form it is used in the fixation of carbon dioxide into organic compounds. This mechanism for energy transfer is the same as that found in heterotrophic bacteria.

In the absence of sulfur, *Thiobacillus thiooxidans* oxidizes an endogenous polysaccharide. LePage and Umbreit¹⁸ have found that this polysaccharide is metabolized via a series of phosphorylated intermediates identical with those occurring in heterotrophs, and O'Kane has established that this bacterium can carry out the autotrophic synthesis of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and biotin, all growth factors for heterotrophic bacteria and higher organisms.

It would seem that the metabolism of this autotrophe, and perhaps others as well, might be divided into two phases. In the first, carbon dioxide is fixed and transformed into a few reactive organic compounds by the use of the energy obtained from the oxidation of inorganic substances or from light (in the case of the photosynthetic forms). This would be a unique and distinctive portion of autotrophic metabolism. In the second phase, these few metabolically active substances are used in the synthesis of cellular material by processes comparable to those occurring in heterotrophs.

Although the influence of light on the metabolism of the purple and green sulfur bacteria had been suspected for some time, it was not until 1941 that the photosynthetic character of these microorganisms was first demonstrated through the work of van Niel.¹⁹ The green sulfur bacteria, strictly anaerobic conditions in the presence of hydrogen sulfide and carbon dioxide. In the absence of oxidizable sulfur compounds, green sulfur bacteria will develop in the presence of organic compounds, which are metabolized under conditions. As in plants, photosynthesis in bacteria is accompanied by an oxidation-reduction process, and may be represented by the following equation:



It is to be noted that in the case of green plants, the process of photosynthesis is accompanied by the oxidation of hydrogen sulfide, resulting in the formation of elemental sulfur. In the absence of hydrogen sulfide, green plants are unable to grow.

¹⁸ LePage, Arch. Biochem. 1942, 25 617.
¹⁹ See the references for van Niel, J. Biol. Chem. 1941, 137 113.

least 15 months. It can be grown on the chorio-allantoic membrane of the developing chick embryo. It differs from the virus of infectious hepatitis and from the mouse hepatitis virus described by Gledhill and Andrewes (see below).

INFECTIOUS HEPATITIS IN ANIMALS

Hepatitis of viral origin seems to be not uncommon in animals. Among dogs and mice we know that the disease may be widely spread under natural conditions, and there is reason to believe that the same may be true of certain other animals.

Infectious hepatitis of Dogs.—Rubarth (1947) described an infectious hepatitis of dogs that appears to be widespread in Sweden. The disease is sometimes known by his name. All breeds are affected, but terriers seem to suffer most. Susceptibility is greatest just after weaning. The clinical picture is one of apathy, anorexia, thirst, diarrhoea and vomiting, fever and occasionally convulsions; jaundice is uncommon. The disease, which is contagious, ranges in severity from the acute fulminating type with death in 24 hours to a completely symptomless infection. On the whole, the case-fatality rate seems to be low. *Post mortem* in fatal cases hepatitis and splenitis are found with increase of fluid in the peritoneal cavity and subcutaneous tissues. Histologically, Rubarth reported the presence of intranuclear inclusions in the liver cells and in the vascular endothelium. He prepared a freeze-dried antigen from the liver which fixed complement with the serum of affected dogs. From the fact that 70 out of 100 dogs of various ages contained complement-fixing antibodies in the blood, he concluded that the disease was very common but was not usually diagnosed. He was able to reproduce the disease by the intraperitoneal inoculation of liver, serum or peritoneal fluid into susceptible puppies, but not into laboratory animals; the incubation period of the experimental disease was 3 days. Liver suspensions were still infective after Seitz filtration. Rubarth's observations have been generally confirmed by workers in other countries (see Parry *et al.* 1951). Miles and his colleagues (1951), who studied an outbreak in Great Britain, described the cultivation of the infective agent in the yolk sac of fertile eggs with reproduction of the disease in dogs after 12 successive passages. Recovery from the natural disease is followed by immunity lasting for at least a year. No satisfactory form of treatment is yet known.

Encephalitis of Foxes.—We include here a disease which according to Rubarth (1947) is caused by a virus closely allied to, if not identical with, infectious hepatitis of dogs. Encephalitis of foxes is endemic in certain parts of the United States, and at times assumes epidemic proportions. It is caused by a filtrable virus which invades the blood, probably through the respiratory mucosa, and may be demonstrated in various tissues. Clinically the disease is characterized by anorexia, hyperexcitability or lethargy, convulsions, paralysis, and not infrequently by death. Pathologically, hæmorrhages in the brain and cord and in the viscera constitute the most striking feature of the disease; perivascular infiltration with round cells is common in the cerebral nervous system, and the meninges are usually involved. Characteristic intranuclear inclusion bodies are found in the ependymal and endothelial cells of the central nervous system. The disease can be reproduced by intramuscular, intranasal, or intracranial inoculation of young foxes with homogenized brain and spinal cord. Unlike the natural disease, which seldom has a case fatality exceeding 20 per cent., the experimental disease is fatal in about 70 per cent. of cases. Infection can also be transmitted to dogs,

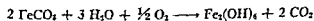
systems. It is suggested by van Niel that the photochemical decomposition of water is the light reaction, while the reduction of carbon dioxide is a dark reaction. The green pigment, known as *bacteriochlorin* or *bacteriochlorophyll*, is a pyrrol pigment with a porphin nucleus containing magnesium, and it is closely related to, though not identical with, green plant chlorophylls *a* and *b*.

A group of organisms known as the non-sulfur purple bacteria contain *bacteriochlorophyll* and are photosynthetic, but differ from the purple sulfur bacteria in that the reduction is coupled only with the oxidation of organic matter, i.e., they are not facultative autotrophes. They are, therefore, intermediate between the sulfur forms and non-photosynthetic bacteria with a typical oxidative metabolism. Available information concerning them has been reviewed by van Niel.²⁰

The bacterial oxidation of sulfur and its compounds is of considerable importance in the processes involved in the maintenance of the sulfur cycle. As in the case of nitrogen, many bacteria are capable of reducing sulfur—the evolution of hydrogen sulfide from decomposing organic matter reflects the widespread reduction of sulfur in lieu of oxygen under anaerobic conditions—but only a few are able to oxidize it. These organisms assume considerable practical significance at times, producing corrosion of metals laid in sulfur-containing clay soils²¹ and are sometimes responsible for the disintegration of stone and mortar.²²

The relative simplicity of the respiratory mechanisms of the sulfur bacteria is in itself of no small general physiological interest, but equal importance attaches to the fact that these organisms appear to constitute a link between the autotrophic organisms and those dependent upon preformed organic matter through the species that are facultatively autotrophic. Their efficiency is of the same order as that of the nitrifying bacteria, i.e., 5 to 8 per cent of the energy made available through the oxidation of sulfur and sulfur compounds is utilized in synthesis of cell substances.

The Iron Bacteria. Certain of the filamentous bacteria are characterized by deposits of ferric hydroxide in the sheath surrounding the filament or, sometimes, within the cell itself. It was early suggested that these organisms are able to oxidize ferrous to ferric iron and utilize the energy so liberated. It appears, however, that although some of the so-called iron bacteria are obligate autotrophes which are dependent upon the oxidation of iron as a source of energy, the presence of ferric hydroxide is not necessarily indicative of an autotrophic metabolism. One species, *Spirillum ferrugineum*, is an obligate autotrophic organism which grows in inorganic solutions containing ferrous carbonate. The oxidation proceeds according to the following reaction:



The reaction is exothermic but the energy yield is small, about 15 calories per mol, and large amounts of ferrous salt must be oxidized. Starkey²³ has shown that the oxidation of 55.8 gm. of iron as ferrous carbonate produces 106.8 gm.

²⁰ van Niel *Bact. Rev.*, 1944, 8.1.

²¹ Cf. Bunker *Jour. Soc. Chem. Ind.*, 1939, 58.93.

²² Gistl *Centralbl. f. Bakt., Abt. II, Orig.*, 1940, 102 486.

²³ Starkey *Science*, 1945, 102 532.

lamb, lamb to lamb, and lamb to mouse by the bites of mosquitoes of the *Eretma. podites chrysogaster* group. The period taken for the mosquitoes to become infectious when kept at 30° C. was 19-20 days.

Experimentally the disease can be transmitted to susceptible animals, including mice, monkeys and ferrets, but not guinea-pigs or rabbits, by cutaneous, subcutaneous, or intranasal inoculation. Mice are peculiarly susceptible, most of the animals dying in 36-96 hours after inoculation (Findlay 1932). By continued passage through the mouse's brain a neurotropic variant of the virus was developed (Mackenzie *et al.* 1936).

The virus is widely distributed in the body, and can pass through the placenta to the foetal organs. Its probable size is 23-35 m μ (Broom and Findlay 1933). In blood it is destroyed by exposure to a temperature of 56° C. for 40 minutes, but it survives contact with 0.5 per cent. phenol at 4° C. for 6 months. In blood serum kept in the refrigerator it remains alive for at least 2 years; such stability is probably unique (Smithburn *et al.* 1949b). It can be cultivated in a minced chick embryo medium (Mackenzie 1933). An attack of the disease confers a high degree of immunity in sheep, and the serum of convalescent animals and human beings contains strong neutralizing and complement-fixing antibodies. Mice can be successfully immunized by a virus treated with formol or inactivated photo-dynamically with methylene blue (Mackenzie 1935).

YELLOW FEVER

Little was known about the ætiology of this once-dreaded disease till the studies of the American and the French Commissions were undertaken in the early years of this century.

The American workers, Reed, Carroll, Agramonte and Lazear (1900-01), at the beginning of the century, first showed that the blood of patients was infective during the first 3 days of the fever; that the infecting agent was able to pass through a Berkefeld filter; that the virus was destroyed in the blood by heating to 55° C. for 10 minutes; that infection was not spread by contagion; that mosquitoes, *Aedes aegypti* (also called *Aedes argenteus*, and *Stegomyia fasciata*) fed on patients during the first 3 days of the fever, became, after an interval of about 12 days, capable of transmitting the disease to normal persons; and that by the suppression of these insects, yellow fever could be brought under control.

Marchoux, Salimbeni and Simond (1903), likewise working with human volunteers, largely confirmed these results. They found, moreover, that the serum of patients during the first 3 days of the fever, but not later, proved infective to normal persons when injected subcutaneously in a dose of 0.1-1.0 ml.; that defibrinated blood kept at 24°-30° C. under a vaseline seal remained infective for 5, but not for 8 days; that the injection of blood kept for 8 days under these conditions was apparently able to produce some degree of immunity in human beings; and that the serum of convalescent patients appeared to be endowed with both prophylactic and therapeutic properties.

The natural outcome of these findings was to undertake an intensive anti-mosquito campaign. The result of this so far exceeded expectations that yellow fever was apparently wiped out of Central and South America. For a time it was believed that the only serious remaining focus of infection was in West Africa. Further work, however, carried out to a considerable extent under the auspices

of ferric hydroxide but only 0.209 gm. of organic cell material, and points out that it is difficult to establish with certainty the reactions whereby the bacteria precipitate the hydroxide. Some of the iron bacteria are also capable of oxidizing manganese salts and precipitate manganese hydrates in their cells.

Other iron bacteria, however, can live without iron and are able to utilize either ferrous carbonate or soluble iron salts of organic compounds. Still others use organic iron compounds but not inorganic iron salts. Harder²⁴ divides the iron bacteria into three physiological groups.

(1) Those, such as *Spirophyllum ferrugineum*, that precipitate ferric hydroxide from solutions of ferrous carbonate and use the carbon dioxide liberated and the energy produced during the oxidation for their life processes (obligate autotrophes),

(2) Those, such as *Leptothrix ochracea*, that do not require ferrous carbonate but that cause the deposition of ferric hydroxide when either inorganic or organic iron salts are present (facultative autotrophes), and

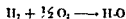
(3) Those, probably including a number of the lower or ordinary water and soil bacteria, that attack organic iron salts, using the organic acid radical as food and precipitating ferric hydroxide or basic ferric salts, which are gradually changed to ferric hydroxide (heterotrophes).

The best known of the iron bacteria are the *Spirophyllum* and *Leptothrix* species indicated above and *Crenothrix polyspora*. These organisms sometimes grow in the conduits of certain public water supplies, where they form unpleasant-looking, brownish, flocculent masses, often leading to complete stoppage of the pipes. The frequent appearance of detached portions of the growth in tap water may give rise to consternation among the water consumers, as in the famous "water calamities" in Berlin, Lille, Rotterdam and other places. There is no evidence that such organisms are directly harmful.

There seems little doubt that iron-depositing bacteria have played an important part in the formation of iron ore deposits, but the relative share of chemical and biological processes in an individual case can be determined only by a thorough study of conditions at the time of deposition, especially as regards sedimentation, climate, depth of water, nature of material in solution and other factors.²⁴

The Oxidation of Hydrogen. The bacterial oxidation of hydrogen has already been indicated in the case of the oxidation of hydrogen sulfide to elementary sulfur. Other organisms, given the generic name *Hydrogenomonas*, are, however, facultative autotrophes which depend upon the oxidation of hydrogen as a source of energy when grown under autotrophic conditions. The hydrogenase is not formed in organic media, but the bacteria grown in inorganic solutions respire with both hydrogen and organic compounds as substrates, suggesting that the hydrogen-oxidizing catalytic system is independent of the normal respiratory process.²⁵

The first of these organisms was described in 1906 by Kaserer and named *Hydrogenomonas pantotrophia*. Other species were described later, such as *Hydrogenomonas vitrea* and *Hydrogenomonas flava*. The oxidation of hydrogen:



²⁴ Harder: U. S. Geological Survey, Professional Paper 113, Washington, 1919. See the review by Duff, *Pflanzenforschung*, 1934, Heft 16.

²⁵ Kluyver and Manten: *Antonie van Leeuwenhoek Jour. Microbiol. Serol.*, 1942, 5: 71.

the disease for the rest of their lives. It was shown that the bite of a single mosquito was sufficient to produce a fatal infection in a monkey. The virus in the circulating blood of monkeys was able to pass through Berkefeld V and N, but not through W, candles.

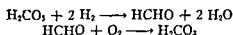
After subcutaneous inoculation of a monkey with infective material, there is an incubation period of 3 to 4 days, followed by a rise in temperature of about 3° F.; this temperature persists for 36 to 48 hours, and then falls suddenly to subnormal; collapse occurs, and the monkey dies about 5 to 6 days after inoculation. *Post mortem*, jaundice is usually present in some part of the body, especially the laryngeal mucosa and the subcutaneous fat; small hæmorrhages are frequently found, particularly in the lungs and alimentary canal, and in the wall of the stomach; dark coffee-ground material is often seen in the stomach and small intestine; the most constant feature, however, is the pale, mottled liver, which microscopically shows fatty degeneration and mid-zonal necrosis. The liver, when fresh, is highly infective for monkeys—in a dose of about 0.000,02 gm. When frozen, the liver remains virulent for about a fortnight; when dried and frozen, it remains virulent for about 3 months (Hindle 1929). This forms a very useful method of preserving the virus. The blood of infected monkeys contains enormous quantities of virus, proving infective sometimes in 1/50–1/100 million.

White mice are also susceptible to infection (see Theiler 1930, Dinger 1931). The intracerebral inoculation of these animals with 0.05 ml. of infective blood from a rhesus monkey gives rise, after an incubation period of 5 or 6 days, to an illness characterized by ruffling of the coat, inactivity, photophobia, paresis of the hind legs, tonic and clonic contractions of the whole body, and finally coma and death in 6–9 days. *Post mortem* there is an acute encephalitis, often with dilatation of the skin vessels, swelling of the lymph glands, hæmorrhages into the stomach, erosion of the gastric mucosa, fatty degeneration of the liver, and enlargement of the suprarenals. Infection can be carried on indefinitely by cerebral passage. According to Findlay and Clarke (1935a), an encephalitis may be set up in both monkeys and mice by the intranasal instillation of a neurotropic strain of virus (see also Findlay and Stern 1935). Guinea-pigs can be infected intracerebrally, provided a neurotropic strain is used (Dinger *et al.* 1930, Mathis 1934, Lloyd and Mahaffy 1935).

Properties of Virus.—Yellow fever is caused by a filtrable virus. Its reported diameter by the gradocol membrane technique is 17–28 μ (Findlay and Broom 1933), and by the ultra-centrifuge technique 12–19 μ (Pickels and Bauer 1940) or 29–31 μ (Polson 1954). Its successful cultivation in mouse embryo tissue was reported by Lloyd, Theiler and Ricci (1936). Jadin (1937) grew it on the chorio-allantoic membrane of hens' eggs; and Elmendorf and Smith (1937) simplified this procedure by direct inoculation of the virus into the chick embryo. When grown for several generations in tissue cultures, its virulence becomes modified; it no longer gives rise to a fatal disease when injected into monkeys subcutaneously, but it remains capable of killing mice inoculated intracerebrally (see Theiler and Smith 1937). Other changes can be produced by animal passage (see Sawyer *et al.* 1930, Findlay and Clarke 1935b, Findlay and MacCallum 1938b). No antigenic differences have been recognized among strains of different origin. The virus contains a soluble antigenic material, which may be demonstrated as a precipitinogen in the serum of monkeys during the height of the illness (Hughes 1933). Survival of the virus in infected blood has already been referred to (see also Sawyer *et al.* 1929). It is killed by heat at 55° C in 5 minutes, and rapidly dies in saline suspensions. In tissue, however, that is frozen solid, it remains alive for some weeks, and in tissue that is frozen and dried it retains its viability for years. It

yields relatively large amounts of energy (34.2 Cal. per gram as contrasted with 4.1 Cal. per gram of starch) and, although it cannot be accurately measured, the free energy efficiency of these organisms appears to be somewhat higher than that of the other autotrophes, i.e., 10 to 20 per cent.

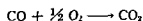
It has been suggested that all the hydrogen is not burnt directly by these organisms but that a part of it is converted to formaldehyde, which may in turn be either oxidized or used for synthesis.



Although most of the hydrogen bacteria are obligate aerobes, some are able to grow anaerobically in the presence of nitrate.

As in the case of the oxidation of iron, the oxidation of hydrogen is not confined to a few species of strict autotrophes, and it has been found that a wide variety of well known heterotrophic bacteria oxidize hydrogen in their respiratory process, as pointed out earlier.

The Oxidation of Carbon Monoxide. The bacterial oxidation of carbon monoxide, comparable in many respects to the oxidation of hydrogen, was reported by Beijerinck and van Delden.²⁶ These workers isolated an organism which they named *Bacillus oligocarbophilus*, an obligate autotrophe which grew in the absence of organic carbon compounds and obtained its nitrogen from ammonia, nitrite or nitrate. It was later shown that this organism derived its energy through the oxidation of carbon monoxide to carbon dioxide.²⁷



HETEROTROPHIC BACTERIA

It is seldom if ever possible to draw sharp lines of demarcation in biology, and the separation of bacteria into autotrophic and heterotrophic forms being no exception, it must necessarily be to some degree dogmatic. It has already been pointed out that some of the sulfur, iron and hydrogen bacteria are facultative autotrophes, i.e., that they may live by oxidation of either inorganic or organic compounds. Such forms may well be regarded as connecting links between the two physiological types. A somewhat different kind of interconnection is the sharing, between obligate autotrophes and obligate heterotrophes, of common metabolic mechanisms, such as the utilization of gaseous hydrogen, the assimilation of carbon dioxide and the phosphorylation of carbohydrates. Clearly, then, the distinction between the two physiological types is not a sharp one even though the concepts autotrophe and heterotrophe remain extremely useful.

CARBOHYDRATE METABOLISM

Carbohydrate is the chief energy source for almost all heterotrophic bacteria, The functional equivalence of both aerobic and anaerobic metabolism of

²⁶ Beijerinck and van Delden: *Centralbl. f. Bakt.*, 1903, Abt. II, 2:33.

²⁷ Distilled water standing in the laboratory for long periods may acquire immunologic properties presumably owing to the growth of bacteria. Organisms able to develop on ammonia and carbon monoxide absorbed from the laboratory air may, in some cases, account for such growth on distilled water, a medium which might be supposed to be entirely lacking in nutriment.

passage through the mouse's brain, together with human serum. An attenuated mouse passage virus, without serum, was recommended by Sellards and Laigret (1932, 1936); but though it has been used extensively on African natives, it has been generally regarded as too dangerous for human use. The vaccine developed by Peltier (1917), which is prepared by drying mouse brains infected with a neurotropic strain of yellow fever virus, is open to the same criticism; large numbers of cases of encephalitis followed its use in Eastern Nigeria, many of them fatal.

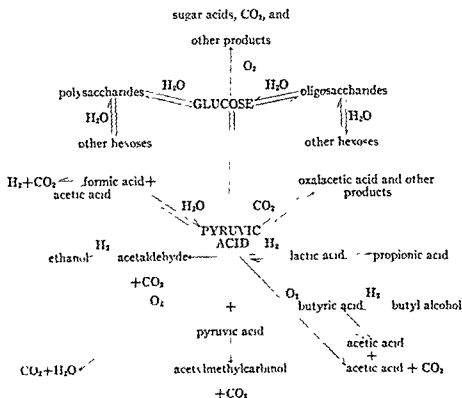
Much the most satisfactory vaccine so far produced is one made with the variant strain, 17D. This was developed by American workers by growth of the virus in tissue culture containing first mouse embryonic tissue, then whole chick embryo, and finally chick embryo from which the brain and cord had been removed. This strain was found to be greatly attenuated, failing to kill monkeys inoculated either subcutaneously or intracerebrally, and producing fatal encephalitis in mice after an average incubation period of 8 instead of 6 days; it still, however, retained its antigenic potency. Large quantities of vaccine could be prepared by inoculation of the variant strain into the developing chick embryo, incubating the eggs for 3-4 days, grinding up the embryo, suspending it in human serum, and clearing, freezing and drying the resulting mixture. For use the dried virus was rehydrated. The dosage for man was calculated according to the number of mice dying after intracerebral inoculation (Theiler and Smith 1937, Smith *et al.* 1938, Bugher and Smith 1944). This vaccine has been widely used, with excellent results. In the early days occasional batches gave rise to jaundice, coming on as a rule 6 weeks to 4 months after inoculation (see Fox *et al.* 1942b). During the 2nd world war no fewer than 28,585 cases of jaundice occurred in the U.S. Army between January and July, 1942, out of about $2\frac{1}{2}$ million troops inoculated; 62 of these cases proved fatal (Report 1942a, b). There is little doubt that the jaundice was due, not to the yellow fever virus, but to the human serum used in the preparation of the vaccine (see p 2207). Hargett, Burruss and Donovan (1943) later prepared a similar vaccine from which the serum was omitted. No jaundice has so far followed the use of this "aqueous base" vaccine; though Fox and his colleagues (1942a) recorded a number of encephalitic reactions among those inoculated, which they attributed to variation in the 17D strain itself. Kept in the ice-chest the desiccated 17D chick embryo vaccine remains active for at least a year. Judged by the persistence of neutralizing antibodies in the serum, immunity following vaccination lasts for at least 5 years (Anderson and Gast-Galvis 1947). The danger that vaccinated persons may serve to infect mosquitoes seems to be negligible, partly because the concentration of virus in the blood is very low, and partly because mosquitoes artificially infected with modified viruses of this type are unable to transmit the disease to susceptible animals (Roubaud *et al.* 1937, Smith *et al.* 1938, Whitman 1939). For an account of the aetiology, epidemiology and prevention of yellow fever, the reader is referred to articles by Sellards (1941), Russell (1941) and Gordon (1941) and to the authoritative monograph by Strode and his colleagues (1951).

DENGUE FEVER

Dengue fever is a disease of warm climates, and is peculiar to man; it occurs during the summer months, often in severe epidemic form, attacking a high proportion of the exposed population, and it is followed by a comparatively short-lived

carbohydrate was first recognized by Pasteur, and it is now evident that, although bacteria form many substances from carbohydrate, the breakdown of carbohydrate proceeds in general along well defined pathways, and variations among species are not so great as was once thought. The chief modes of carbohydrate breakdown are summarized in the accompanying diagram.

CARBOHYDRATE BREAKDOWN IN BACTERIA



Breakdown of Oligosaccharides and Polysaccharides. Some *Pseudomonas* species can rapidly oxidize lactose and maltose to the corresponding aldobionic acids,²⁸ but most bacteria split compound sugars to their component monosaccharides before they are further metabolized. Oligosaccharides and polysaccharides may be hydrolyzed by the addition of a molecule of water to each glycosidic linkage. The hydrolysis of a compound sugar is an irreversible reaction and proceeds with the liberation of energy, but the energy yield is very small in comparison with that made available through oxidation of the component monosaccharides. Bacteria contain enzymes which are capable of hydrolyzing a wide variety of polysaccharides and oligosaccharides.

Cellulose.²⁹ Although cellulose is the most widely distributed polysaccharide in nature, the ability to bring about its decomposition is possessed by only a few organisms. The enzyme cellulase is found in a few higher organisms, some snails and a few marine forms, but even the herbivorous animals are dependent upon the presence of cellulose-decomposing microorganisms in the intestine for

²⁸ Stodola and Lockwood: *Jour. Biol. Chem.*, 1947, 171 213.

²⁹ See review by Waksman: *Bot. Rev.*, 1940, 6 637.

antigenic types of virus, and that immunity is restricted to the homologous type (Sabin 1952, Meiklejohn *et al.* 1952).

In the diagnosis of dengue the severe leucopenia, with a proportional increase in the immature granulocytes, is of help. According to Hotta (1952) a skin reaction can be elicited from the 2nd day onwards by the intradermal injection of an antigen prepared from infected mouse brain. (For a general account of the disease, and of the investigations carried out during the 2nd world war, see Simmons 1941, Lumley 1943, Yaoi and Arakawa 1948, and Sabin 1952.) In control of the disease, the usual measures against mosquitoes should be employed.

SANDFLY FEVER

SYNONYM: Phlebotomus Fever.

This disease occurs in tropical and sub-tropical countries. It is characterized by an incubation period of 1-6 days, and a fever lasting at least 3 days, accompanied by severe headache, pains in the back, bones, joints, and muscles, and followed by considerable prostration. It is rarely fatal. Second, and even third, attacks do occur, but are not very common. The causative agent appears to be a filtrable virus. There is, however, some discrepancy of opinion on its properties. For instance, Shortt and his colleagues (1938) find that it is about 160 μ in diameter, and that it can be grown in a tissue culture medium containing minced chick embryo, human serum and chick plasma, and on the chorio-allantoic membrane of the developing chick embryo (Shortt, Rao and Swaminath 1936). Sabin, Philip and Paul (1944), on the other hand, maintain that it is only 20-40 μ in diameter and that it cannot be cultivated by the chorio-allantoic membrane technique. Again, according to Shortt and his colleagues (1940), the virus can be demonstrated in the patient's blood for a week in most cases, and occasionally for as long as 6 weeks. The time during which the serum is infective is given by the American observers as 3 days, namely for 1 day before to 2 days after the onset of the fever. Sera kept in the ice-chest remain virulent for months. As present in human blood, the virus, when inoculated into *rhesus* monkeys, gives rise to a mild fever; but the cultivated virus produces only a symptomless infection (Shortt *et al.* 1940). The incubation period after intracutaneous injection into human beings is said to be 2½ to 6 days (Sabin and Paul 1944). Neutralizing antibodies appear early in the serum of convalescent patients and of inoculated monkeys; they can be demonstrated by monkey protection tests, or by inhibition of the growth of the virus in culture. Immunity is said to persist for at least 4 months. Infection is spread by the sandfly *Phlebotomus papatasi* (see Whittingham 1924), though possibly other species may act as vectors. The flies do not become infective for 7-10 days after biting a patient with the fever. Diagnosis is often difficult. The absence of catarrhal symptoms is of help in distinguishing the disease from influenza, and the absence of rigors from malaria. Skin rashes do not occur (Walker and Dods 1941). The disease may closely simulate lymphocytic choriomeningitis, but the cell count in the cerebrospinal fluid is not increased (Pearson 1941). In the blood there is a leucopenia. Prophylaxis consists mainly in abolishing sandfly breeding grounds, spraying with D.D.T., and in sleeping under a close-mesh net. Dimethyl phthalate and a pyrethrum vanishing cream are said to afford considerable protection against sandfly bites (Sabin and Paul 1944).

the preliminary decomposition of this polysaccharide.³⁰ The breakdown of cellulose and other polysaccharides in nature is an essential part of the decomposition of plant residues and the consequent returning to the atmosphere of carbon dioxide removed through the photosynthetic activities of chlorophyll-containing plants. Relatively few species of bacteria are able to decompose cellulose, but those that do are widely distributed in the soil and the mud of sea bottoms as well as in the intestines of herbivorous animals.

A number of bacteria, both aerobic and anaerobic forms, have been isolated which decompose cellulose actively in pure culture. Perhaps the best known aerobic organism is a spiral form, *Spirochaeta cytophaga*, which occurs in soil in considerable numbers. A large number of other bacteria which bring about the aerobic decomposition of cellulose have been studied, but the identity of many of them is doubtful. The breakdown of cellulose by anaerobic bacteria is ordinarily accompanied by further decomposition of the glucose so formed to hydrogen and methane in some cases and to organic acids and carbon dioxide in others. Some species have been described, such as *Bacillus cellulosa dissolvens*, which was isolated from human feces, but in general these organisms are not well known. Equally obscure are the thermophiles which have been isolated from actively fermenting manure heaps. The decomposition of cellulose in nature is largely, if not entirely, a process brought about by mixed cultures of bacteria.

The hydrolysis of cellulose takes place in two stages: first, a breakdown to the disaccharide cellobiose; and second, the hydrolysis of cellobiose to glucose. These reactions are brought about by two separate enzymes, cellulase and cellobiase respectively, and the decomposition, to cellobiose at least, takes place outside the bacterial cell.

Hemicelluloses and Pectins. In addition to cellulose proper, plant tissue contains other polysaccharide material designated as hemicellulose and pectin. On hydrolysis, both yield pentoses, hexoses and uronic acids. Although hemicelluloses are decomposed in nature, largely under the influence of bacteria present in the soil, the chemistry of these compounds is largely unknown and, in consequence, little is known of the processes of their decomposition.³¹

The decomposition of pectin, through the agency of the enzyme pectinase, is brought about by a comparatively small number of species of anaerobic bacteria. The process itself is of considerable commercial importance since these organisms are responsible for the retting of flax and hemp and for the rotting of certain fruits and vegetables. Retting is accomplished by submerging flax and hemp in water and allowing the plant tissue to remain until the dissolution of pectin allows the separation of the fibers.³² The organisms responsible for the decomposition of pectin are closely related to the group of anaerobic soil forms, the amylobacter group, which includes the butyric acid organisms and others. A number of other bacteria, including *Lactobacillus*, *Aerobacillus*, *Micrococcus* and *Enterococcus*, decompose pectins.³³ The nature of the process

³⁰ Hastings Bact. Rev., 1944, 8.235.

³¹ See Waksman *Humus*. Williams and Wilkins Company, Baltimore. 1936.

³² Fuller and Norman. Iowa Agr. Expt. Sta. Res. Bull., 1946, 343:893, *ibid.*, 1946, 344:925.

³³ Werch et al. Jour. Inf. Dis., 1942, 70:231.

convalescent serum is said to be of some value (see Report 1951a, Beard 1952, Andrew 1953). (See also Report 1953b, Powell 1954.)

DISTEMPER

Distemper is an acute infectious disease of young carnivorous animals. It is very common in puppies, and has given rise to great trouble in foxes reared for the fur industry. In the dog the disease is characterized by an incubation period of 3-6 days, an initial coryza, fever of a peculiar type, severe gastro-intestinal disturbance, and catarrhal inflammation of the respiratory tract often passing on to pneumonia. Encephalitis is a less common complication. The disease normally lasts 2-4 weeks and has a low mortality; but complicated cases, which probably constitute 40 or 50 per cent. of all cases, may last for several weeks and are accompanied by a case-fatality rate running up to 80 per cent. The disease is said to be characterized by acute anaemia and hypoglycaemia (Wharton and Wharton 1934).

The nature of the *encephalomyelitis* that is sometimes observed has given rise to a great deal of discussion. Perdrau and Pugh (1930) regarded it as being caused by a virus different from the distemper virus, mainly because of the demyelination that is present. Verluise (1939), however, recognized two types of encephalitis, one caused by the distemper virus itself, and another by a neurotropic virus that was able to produce encephalitis in the absence of distemper. In England, Macintyre, Trevan and Montgomerie (1948) likewise recognized two types of encephalitis caused by a filtrable agent infective for the ferret, namely the distemper virus and the virus of hard pad disease.

In *hard pad disease* the foot pads of the dog become hard and often very thick, usually within a week of the onset of illness. Convulsions and other signs of encephalitis develop. Post-mortem examination reveals demyelination of the cerebellar peduncles, glial proliferation, and weakly acidophilic intranuclear inclusion bodies. A suspension of infective material from hard pad disease injected into the ferret gives rise to a disease resembling distemper, and into the dog merely to a febrile reaction lasting 10-14 days. According to Macintyre and his colleagues animals that have recovered from hard pad disease are not immune to distemper, nor does distemper antiserum protect against hard pad disease.

These conclusions are somewhat at variance with the findings of Australian and American workers (Hurst *et al.* 1943, Koprowski *et al.* 1950), both of whom isolated what appeared to be the true distemper virus from dogs suffering from encephalitis. Demyelinating lesions were found in the cerebellum, and acidophilic, mainly intranuclear, inclusion bodies in the glial cells. Injected into puppies, the virus produced a nervous type of distemper, but in ferrets vaccinated against distemper it produced no disease at all. Mansi (1951), who isolated a strain of virus from a dog suffering from encephalitis in Glasgow, found it to be immunologically identical with the true canine distemper virus and the hard pad virus of Macintyre, Trevan and Montgomerie. He would regard it as a distemper virus endowed with neurotropic affinities. Though it is too early to reach any definite conclusion, it seems probable that the distemper virus has variants, some of which exhibit a special predilection for the central nervous system. Of these, the hard pad virus may be one.

Distemper occurs naturally in the ferret, and is characterized by an incubation period of about 10 days, an initial coryza, and the formation of vesicles and pustules around the mouth; just as in dogs, encephalitis may develop in a small proportion of cases. Distemper in the ferret is a very fatal disease, causing a mortality of about 90 per cent. (Dunkin and Laidlaw 1926). The disease has been studied to a limited extent in the cat (Hindle and Findlay 1933, Dalling 1934), the fitch

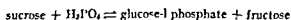
of decomposition is obscure, though the sugars resulting from hydrolysis are further decomposed to the usual products of sugar oxidation. Few if any of the pectin-decomposing bacteria are able to hydrolyze cellulose also. The combination of the two properties in a single organism would, of course, result in the decomposition of the flax fibers freed by hydrolysis of the pectin.

Starch. Unlike cellulose, starch may be hydrolyzed by a wide variety of living organisms, and the extracellular enzyme diastase, or amylase, is possessed by many bacteria. Like cellulose, however, starch is hydrolyzed in two stages, first to the disaccharide maltose, and second by a further hydrolysis to glucose. The ability of a given organism to hydrolyze starch has acquired an importance equal to sugar fermentations as a differential character in the study of some bacteria.

Miscellaneous Polysaccharides. A wide variety of gums and mucilaginous substances, polymers designated as pentosans, levans, galactans, and the like according to the nature of the products of their hydrolysis, may be decomposed by bacteria. Polysaccharides of bacterial origin, such as pneumococcus polysaccharide, may be decomposed by other bacteria (p. 394), and bacteria have been found which hydrolyze agar, a polysaccharide resistant to the action of the great majority of bacteria.³⁴

Polymeric Degradation. Leibowitz and Hestrin³⁵ have described a mode of compound sugar degradation which is of wide occurrence in bacteria. For example, *B. subtilis* decomposes sucrose with the liberation of free glucose and the formation of levan, a fructose polysaccharide. This method of sugar breakdown has been termed polymeric degradation and involves the synthesis of a new glycosidic bond at the expense of a previously existing one. The reaction appears to be reversible and may be responsible for the breakdown or synthesis (*vide infra*) of sucrose and polysaccharides.

Phosphorylysis. Polysaccharides and oligosaccharides may also be split, not by the addition of water but by the addition of phosphoric acid instead. Such a cleavage is called phosphorylysis and is catalyzed by a group of enzymes called phosphorylases. The sucrose phosphorylase of *Ps. saccharophila* catalyzes the reaction³⁶.



In contrast to hydrolysis, phosphorylysis of glycosidic bonds is reversible, and compound sugars may be synthesized by the reverse reaction (*vide infra*).

Anaerobic Breakdown of Glucose and Other Hexoses. In most bacteria the first step in the metabolism of the six-carbon sugars is their cleavage to two three-carbon compounds. This process, referred to as either fermentation or glycolysis, proceeds without the intervention of molecular oxygen, although it occurs in many organisms under both aerobic and anaerobic conditions. Strictly speaking, the term *fermentation* is best used to describe all the metabolic transformations of carbohydrate which occur without direct reaction with molecular oxygen, and the term *glycolysis* is best limited to the breakdown of

³⁴ See Stanier, *Jour. Bact.*, 1941, 42:527.

³⁵ For references to original papers, see Barker and Doudoroff, *Ann. Rev. Biochem.*, 1946, 15:497.

³⁶ See the review by Doudoroff: *Fed. Proc.*, 1945, 4:241.

given up (Dalling 1932b). If, however, a fully virulent dried ferret virus is inoculated, followed 1-2 hours later by a dose of immune serum, a satisfactory immunity results. This method is extensively used in practice in order to save the veterinarian two visits. For treatment of the disease immune dog serum may be used. It is said to be of considerable value if given within 7 days of the onset of symptoms (Wright 1932). It is also useful for prophylaxis.

INFECTIOUS FELINE AGRANULOCYTOSIS. FELINE ENTERITIS. FELINE PNEUMONIA

A widespread highly contagious disease of cats occurs in the United States to which the name feline enteritis is often given, but for which the term infectious feline agranulocytosis is preferred by American workers. It was studied by Hammon and Enders (1939) and by Syverton and Lawrence and their colleagues (Syverton *et al.* 1943, Lawrence *et al.* 1943). Though the accounts of these observers differ to some extent, there is little doubt that they were dealing with the same disease. Kittens and young cats seem to be mainly affected. The incubation period is 4-7 days. The symptomatology is irregular, but listlessness, prostration, anorexia, vomiting, and diarrhoea are often present. The temperature is raised, and nasal and ocular discharges are common. The most characteristic feature, however, is the occurrence of a profound leucopenia, amounting sometimes to almost complete absence of white cells from the circulating blood. Death usually occurs in 2-4 days. The case fatality is given as 50 per cent. At post-mortem there is little macroscopic evidence of disease; but microscopically there is severe aplasia of the cellular elements of the bone marrow, and conspicuous intranuclear inclusion bodies are found in the epithelial cells of the intestinal mucosa and the reticular cells of the spleen and lymph nodes. Infection is spread by contact with infective discharges. A virus, capable of passing through a Berkefeld W filter, and therefore probably not greater than 35 μ in diameter, can be demonstrated by animal inoculation in the blood, organs, nasal secretion, faeces, and sometimes urine of infected animals. The disease can be reproduced experimentally by inoculation of non-immune animals by the oral, intranasal, subcutaneous, and intravenous routes. Attempts to cultivate the virus have so far failed. The virus is specific for cats, and is apparently distinct from the feline distemper virus, but further observations are required. Recovery from the disease is accompanied by the appearance of specific neutralizing and protective antibodies.

According to Jennings (1947, 1949) feline enteritis is not the same as feline agranulocytosis. Jennings recognizes a third disease—feline pneumonia. Enteritis is said to affect young animals in particular: pneumonia affects cats at all ages. Whether each of these three diseases is caused by a distinct virus is, however, doubtful, and further work is required to clear up the present confusion.

SWINE FEVER

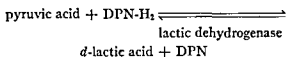
SYNONYMS: Hog Cholera. *Schweinepest* (German). *Peste du porc* (French).

Swine fever or hog cholera is an infectious disease, peculiar to pigs, caused by a filtrable virus. It is widespread throughout the world; a special form of the disease was described by Montgomery (1921) in East Africa. The disease is primarily a septicæmia, but in a large proportion of cases intestinal compli-

cifically, Utter and Werkman⁴⁰ and Still⁴¹ have shown the occurrence of almost all the reactions of the Meyerhof-Embden-Parnas-Cori-Warburg scheme in the fermentations of the coliform bacteria.

While most bacteria convert glucose to pyruvic acid by the same mechanism, they show great diversity in the manner in which they dispose of pyruvic acid once it is formed, thus giving rise to the different types of bacterial fermentations. Under anaerobic conditions, pyruvic acid or one of its derivatives is reduced by accepting electrons, usually from 3-phosphoglyceraldehyde, in a coupled oxidation-reduction reaction.

Lactic Acid Fermentation. The simplest type of fermentation is the lactic acid fermentation in which pyruvic acid itself is the electron acceptor.

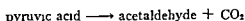


The overall equation for the lactic acid fermentation is:

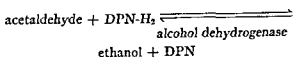


Bacteria of the genera *Lactobacillus* and *Streptococcus* form large amounts of lactic acid from glucose, and some species have an almost pure lactic acid fermentation. In addition, lactic acid is one of the products of glucose fermentation in many other bacteria.

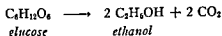
Alcoholic Fermentation. In alcoholic fermentation, pyruvic acid is decarboxylated to acetaldehyde by pyruvic carboxylase (in yeast, a Mg-diphosphothiamine-protein)



The acetaldehyde then functions as the electron acceptor in the oxidation-reduction reaction of glycolysis and is reduced to ethanol.

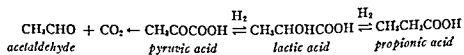


The overall equation for alcoholic fermentation is:



Some *Sarcina* species carry out an almost pure alcoholic fermentation, and many other bacteria produce small amounts of ethanol from glucose.

Propionic Acid Fermentation. The propionic acid bacteria, which are very closely related to *Lactobacillus*, reduce pyruvic acid all the way to propionic acid. They also decarboxylate pyruvic acid to acetaldehyde and CO_2 .



⁴⁰ Utter and Werkman: *Jour. Bact.*, 1941, 42:665; *Biochem. Jour.*, 1942, 36:485.

⁴¹ Still: *Biochem. Jour.*, 1940, 34:1177, 1374.

inactivated with crystal violet. The vaccine, which was developed by McBryde and Cole (1936), consists essentially in the use of defibrinated blood taken from infected animals, and inactivated with crystal violet in a final concentration of 1/2,000; ethylene glycol is added to keep down bacterial contamination (Doyle and Wright 1917). A single dose of 5-10 ml. is given subcutaneously according to the weight of the animal, or a smaller dose by the intradermal route. Immunity develops within a fortnight of injection and remains for at least a year. The vaccine can be used only on healthy herds that are not in contact with infection. In the United States of America a living lapinized or rabbit-adapted vaccine is preferred. Animals that have been exposed to infection may be passively protected by the injection of antiserum taken from convalescent animals. Simultaneous immunization with antiserum and virulent blood is sometimes undertaken to produce an active immunity under cover of a passive.

Baby pigs may suffer from a very contagious form of *infectious gastro-enteritis* caused apparently by a virus different from that of swine fever. According to Bay, Hutchings,

present in the gastric and intestinal mucosa, and the kidneys show degeneration. The incubation period of the disease is 1-4 days. Infection can be transmitted experimentally to new-born pigs by feeding or injection with minimal quantities of ground-up tissues. Even a 1/1,000,000 dilution of a Berkefeld N filtrate may prove infective by the mouth. In filtrates the causative agent is inactivated by heating to 56° C. for 1 hour, and by exposure to 0.5 per cent. phenol for 30 minutes at 37° C.

RINDERPEST OR CATTLE PLAGUE

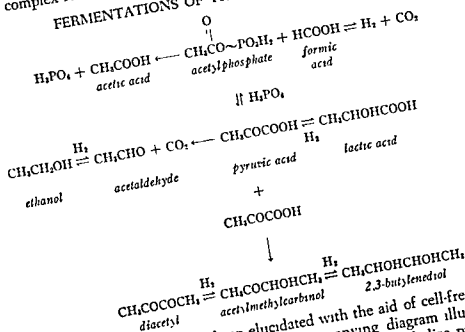
Rinderpest is an acute infectious disease of cattle, characterized by an incubation period of 3-9 days, acute fever lasting 4-7 days, inflammation of the buccal and gastric mucosa often attended by necrosis and ulceration, a persistent leucopenia, and a case-fatality rate ranging from 15 to 75 per cent. The disease is prevalent in southern Europe, Asia, and Africa where it constitutes a major economic problem. It has been imported from time to time into Great Britain, but has always been successfully eradicated by the slaughter policy. The existence of chronic carriers, with diphtheritic ulcers in the abomasum, was recorded by Gibbs (1933). The disease also attacks buffaloes (Schein 1917).

Inoculation of normal animals with tissue fluids from an infected animal reproduces the disease. At the height of the attack the blood proves virulent in a dose of 1/1,000 to 1/25,000 ml. (Schein 1917). There is evidence that the virus is contained chiefly within the leucocytes. Thus in blood that is allowed to clot naturally the serum is avirulent; in defibrinated blood that is centrifuged the deposit alone is virulent; in blood laked with distilled water and centrifuged, the deposit, which consists mainly of leucocytes, alone is virulent (Kolle, see Nicolle and Adil-Bey 1902; Schein 1917, Daubney 1928). Nicolle and Adil-Bey (1902) brought forward evidence to show that the virus, when present in diluted tissue fluids or suspensions, may pass through a Berkefeld N or a Chamberland F candle; the results however are inconstant—possibly because of the intracellular location of the virus. Complement-fixing bodies are present irregularly in the serum of convalescent animals. For laboratory diagnosis reliance is better placed on the

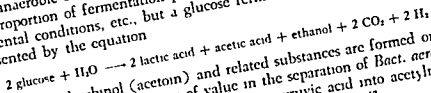
Anaerobic Breakdown of Glucose and Other Hexoses

Fermentation of the Coliform Bacteria.⁴² The coliform bacteria carry out more complex fermentations of glucose than those just described. The details

FERMENTATIONS OF THE COLIFORM BACTERIA



of these fermentations have been elucidated with the aid of cell-free bacterial extracts and isotopic techniques. The accompanying diagram illustrates the manner in which the coliform bacteria, as a group, metabolize pyruvic acid under anaerobic conditions, not all of these reactions occur in any one species. The proportion of fermentation products formed varies with the species, experimental conditions, etc., but a glucose fermentation of *Bact. coli* may be represented by the equation



Acetylmethylcarbinol (acetoin) and related substances are formed only by *Bact. aerogenes*, and this fact is of value in the separation of *Bact. aerogenes* from *Bact. coli*. The enzyme which converts pyruvic acid into acetylmethylcarbinol is a Mg- or Mn-diphosphothiamine-protein complex.⁴³

Butyl Alcohol Fermentations.⁴⁴ Another type of complex bacterial fermentation is carried out by anaerobic organisms such as *Clostridium butylicum*, *Cl. acetobutylicum*, *Cl. welchii* and *Cl. kluyveri*. Pyruvic acid is transformed into acetic acid which condenses with itself to form acetoacetic acid (acetyl phosphate may be an intermediate). Butyric acid and butyl alcohol are then formed by reduction of the acetoacetic acid. In the anaerobic breakdown of glucose by *Cl. butylicum*, fermentation products are formed in approximately the following molar proportions: 50% CO₂, 25% H₂, 12% butyl alcohol, 3% each of isopropyl alcohol, butyric acid and acetic acid, and traces of ethanol.

⁴² For original references, consult Barker and Doudoroff: *Ann. Rev. Biochem.* 1946, 15-497.

⁴³ Silverman and Werkman, *Jour. Biol. Chem.*, 1941, 138-35.

⁴⁴ For discussion of butyl alcohol fermentations, see Barker and Doudoroff: *Ann. Rev. Biochem.* 1946, 15-483.

Attempts have been made to determine whether, independently of aggregation, antibody can retard either the metabolism or the reproduction of bacteria, in a manner similar to the action of the "ablastin" antibody described by Taliaferro for *Trypanosoma lewisi* (see Taliaferro 1948). In the absence of complement, which of course may be bactericidal for bacteria sensitized with antibody, antibody does not decrease oxygen consumption by salmonellae (Sevag and Miller 1948, Harris 1948, Follis *et al.* 1952); with young cultures of *Chr. prodigiosum* and *Sh. flexneri*, indeed, Follis and his colleagues recorded a stimulation of oxygen uptake by specific antibody.

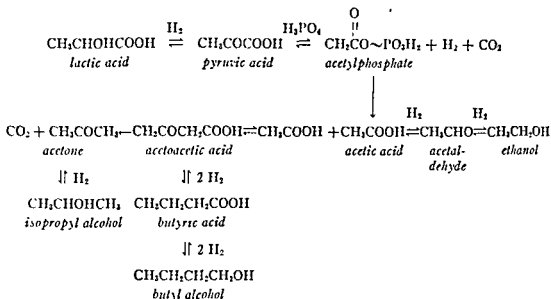
The Availability of Antibody in the Tissues of the Host.

The concentration of antibody in the blood is one of the simplest indications of a state of immunity, and it is possible to determine by experiment the relation between a given blood concentration and a specifically immune state of the animal. This immunity, however, depends on antibacterial qualities not only of the blood, but also on that of the tissues, the intercellular fluids, lymph, and so forth. Moreover, in most natural processes of infection, the defences come into play in the order—tissues, lymphatic system, blood stream, and finally reticulo-endothelial system. Given, then, an animal whose blood contains an adequate concentration of antibody specific for the antigens associated with the virulence of a parasite, we must consider whether the concentration of antibody in other tissues is sufficient for an effective antibacterial response at any of the stages of invasion we have listed. Serum antibodies, whether they result from active or passive immunization, are sooner or later distributed throughout the tissues. They are present in tissue fluids and lymph, but only in very low concentrations in cerebrospinal fluid (see Sohler and Jaulmes 1939).

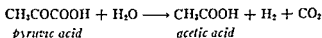
Thus Pagano (1894), Falloise (1903) and Batelli (1904) found that the concentration of hæmolytin was lower in the thoracic lymph than in the blood serum. Hughes and Carlson (1908), working with normal dogs, horses and cats, found that the concentration of hæmolytin for rabbit corpuscles formed a descending series, in the order—serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humour. Becht and Greer (1910) studied the distribution of normal and immune antibodies in dogs, and in addition the rate of transference of antibodies from the circulating blood to the tissue fluids after passive immunization by the intravenous route. Their findings with regard to the distribution of normal and immune antibodies were in accord with those of Hughes and Carlson. In the actively immunized animals they observed a marked rise in the antibody titre of the serum, thoracic lymph and neck lymph, the relative concentrations in the different fluids maintaining the same order as in the normal animals. After passive immunization, the characteristic distribution of antibodies was established within 4½ hours. These findings were confirmed in all essentials by Hektoen and Carlson (1910).

The thoracic duct, however, receives contributions from all the lymphatic channels of the body, including those from the liver, whose lymph ordinarily contains a high proportion of protein, so that the antibody content of thoracic duct lymph may reflect only the permeability of the liver capillaries to antibody. Thus Freund and Whitney (1929) injected agglutinins intravenously into rabbits and found that antibodies accumulated rapidly in liver lymph, slowly in lymph from the leg. Similarly, Becht and Luckhardt (1916) found that cervical lymph had a lower concentration than thoracic duct lymph of intravenously injected antibody. In rabbits actively immunized with diphtheria toxoid, Takahashi (1936) found 0.67 unit of antitoxin per ml. of blood, and 0.29 unit per ml. of popliteal lymph. Nevertheless, with the exception of locally formed antibody (see Chapter 50) the concentration of antibody in tissue fluids and lymph, like that of other serum proteins, tends to be comparatively low. Thus in rabbits actively immunized with the virus of equine encephalomyelitis, the ratio of antibody titres in blood and brain

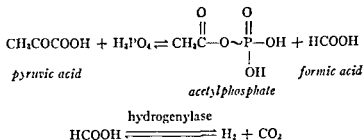
THE BUTYL ALCOHOL FERMENTATION



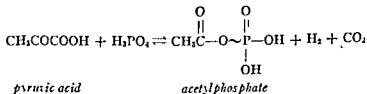
In both of the two complex fermentations described in the preceding paragraphs, pyruvic acid is split to acetic acid, H_2 , and CO_2 by addition of a molecule of water. This reaction is known as the hydroclastic reaction:



However, recent investigations have revealed that the hydroclastic reaction is actually a reversible phosphoryclastic reaction in which acetylphosphate, which contains a high energy phosphate bond, is formed. In *Bact. coli*, the reaction proceeds in two steps:



In *Cl. butylicum*, formic acid is not an intermediate in the formation of H_2 and CO_2 .



Fermentation of Other Sugars, Organic Acids and Alcohols The fermentation of sugars other than hexoses, organic acids and alcohols results in the formation of acids and, sometimes, gases; but, for the most part, the mechanisms involved in these decompositions are unknown. Certain fragmentary ob-

quantities of blood taken from infected animals. Blood, when diluted with saline, and filtered through a Berkefeld or Chamberland candle, still proves infective. By cross-immunity tests in horses and by intracerebral protection tests in mice numerous antigenically different strains of virus can be distinguished (Alexander 1935). The diameter of the virus appears to be about 50 $m\mu$. Prophylactic inoculation may be accomplished by Theiler's method, which consists in the injection of large quantities of immune serum intravenously, together with small quantities of virulent blood subcutaneously, followed by a second serum injection after the appearance of the febrile reaction. More recently, a specially developed neurotropic virus has been used.

INFECTIOUS ANÆMIA OF HORSES

SYNONYM: Swamp Fever.

This disease, which is not to be confused with the swamp fever of human beings caused by *Leptospira grippotyphosa* (see Chapter 82), has a wide geographical distribution, occurring in various parts of Europe, the United States, Canada, Japan, and South Africa. It is most prevalent in low-lying badly drained districts, and reaches its maximum incidence during the summer months. The disease was shown by Vallée and Carré (1904) to be due to a filtrable virus, which is constantly present in the blood stream. By the gradocol membrane technique its size appears to lie between 18 and 50 $m\mu$ (Balozet 1939), and by electronmicrography between 11 and 59 $m\mu$ with an average of 30 $m\mu$ (Reagan *et al.* 1950). Transmission probably occurs by biting flies—*Stomoxys calcitrans*. The disease can be reproduced experimentally by biting insects, but not by contact or feeding; not all workers, however, have been successful with insect transmission (Lemétayer 1949). Equine animals are alone susceptible to the natural disease. Laboratory animals appear to be refractory to experimental inoculation, though it is said that the virus can be established in rabbits by intravenous injection of citrated blood (Reagan *et al.* 1950). The disease may be acute or chronic. The acute form is characterized by fever, extreme weakness, and conjunctival hæmorrhages, the chronic form by general unthriftiness with extreme emaciation. Anæmia does not appear to be a prominent sign. Death may occur in acute cases. Horses may remain carriers for years. There is, as yet, no specific diagnostic test or therapeutic treatment for the disease (for references see Stein 1935).

MALIGNANT CATARRH OF CATTLE

SYNONYM: *Snotsiekte*.

This disease occurs in various parts of the world, and is characterized by fever, catarrh of the oral, nasopharyngeal, and conjunctival membranes, general enlargement of the lymphatic glands, and often symptoms suggesting involvement of the brain or cord. The mortality is subject to considerable variation. Post-mortem examination of fatal cases often reveals petechial hæmorrhages in the stomach, mottling of the liver and kidneys, congestion and petechiæ in the respiratory mucosa, œdema of the lungs, enlargement of the lymphatic glands, and sometimes plate-like extravasations of blood on the surface of the brain. The disease is apparently caused by a virus, which can be transferred to rabbits and back again to cattle (see Daubney and Hudson 1936).

Aerobic Breakdown of Carbohydrate

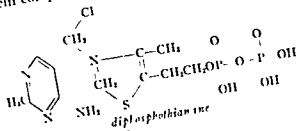
servations are available, such as those of Kay,⁴⁵ who showed that when mannitol, glucose, glucuronic acid, glycuronic acid and saccharic acid are fermented by *Bact coli*, the yields of the more oxidized products (acetic and succinic acids) rise and those of the reduced products (ethanol) fall as one proceeds from the reduced substrate mannitol to the more highly oxidized saccharic acid. The fermentation of glycerol by this same organism has been found⁴⁶ to yield much the same end products as the fermentation of glucose. Similarly, the fermentation of the four-carbon acids, malic, tartaric and fumaric acids, by *Bact. aerogenes* gives rise to the same products as the glucose fermentation⁴⁷, the fermentation apparently proceeds via oxalacetic acid, which is decarboxylated to pyruvic acid (p. 85).

Energy Yields from Anaerobic Breakdown of Pyruvic Acid. Bacterial fermentation of pyruvic acid always results in the liberation of energy, but the diverse nature of the products of fermentation makes exact calculation of the energy changes very difficult. Under proper conditions (see p. 115), many heterotrophic bacteria, such as *Bact coli*, may grow anaerobically with only pyruvic acid, lactic acid or glycerol as the source of energy. The formation of acetylphosphate and the transfer of its high energy phosphate bond to the adenine nucleotides is of frequent occurrence in the bacterial fermentations and represents one possible mechanism of energy conservation and transfer.

Aerobic Breakdown of Carbohydrate. Comparatively little attention has been paid to the aerobic metabolism of carbohydrate in bacteria, and the details of these processes are not well understood. Certain bacteria are able to oxidize such diverse compounds as methane, benzene, phenol, cholesterol, etc.,⁴⁸ but the substrates for most aerobic bacterial oxidations are the hexoses and their anaerobic fermentation products.

Hexoses may be oxidized without preliminary fermentation. Some bacteria which are unable to ferment glucose can oxidize glucose or glucose-6 phosphate with molecular oxygen.⁴⁹ The acetic acid bacteria are noted for the variety of direct oxidation products which they form. These bacteria can oxidize glucose to gluconic acid, 5 ketogluconic acid, and 2-ketogluconic acid.

Direct Oxidation of Pyruvic Acid. Enzymes which catalyze the oxidation of pyruvic acid to acetic acid and CO_2 are formed by many bacteria. Like the enzymes which decarboxylate pyruvic acid to acetaldehyde and CO_2 or convert it to acetyl methylcarbinol and CO_2 , the pyruvic oxidases are diphosphothiamine protein complexes



⁴⁵ Kay, *Biochem Jour.*, 1926, 20 231.

⁴⁶ Brask, *Diss.*, Delft, 1928.

⁴⁷ Barker, *Kon Akad Wet.*, Amsterdam, 1936, 39 1.

⁴⁸ Cf. the review by Zöllner, *Bact. Rev.*, 1946, 10 1.

⁴⁹ Barron and Friedemann, *Jour. Biol. Chem.*, 1941, 137 593.

susceptible animals are brought together. Sheep are more prone to the disease than goats, and—curiously enough—native sheep are more susceptible than pure-bred animals. The disease is characterized by an incubation period of about 2 days, a sudden rise of temperature to 105–108° F., a fall to normal in 2 days followed by obvious illness, diarrhoea with sometimes blood and mucus in the stools, and—in fatal cases—death within 2–4 days of the onset of obvious symptoms. At post-mortem there is an acute hæmorrhagic gastro-enteritis, the spleen is enlarged, the gall-bladder is distended with bile, and the trachea is congested and may contain blood-stained mucus. The case fatality in native sheep is about 70 per cent. The blood is infective during the fever and for a day after its fall. The infectivity is retained after passage through a Chamberland F candle. Though the urine may also contain the virus, infection does not appear to spread by contact, but is transmitted by the bite of the “brown” tick, *Rhipicephalus appendiculatus*. Ticks can be infected in the nymphal as well as the adult stage, and there is some evidence to suggest that hereditary transmission of the infection may occur. Immunity to the disease persists for, at any rate, some months. No method of artificial immunization has yet proved satisfactory. The “brown” tick is also responsible for carrying East Coast fever in cattle. Dipping cattle at a 3-day interval will help to clear the ground of ticks, and so diminish the risk to sheep of infection with the virus of the Nairobi disease.

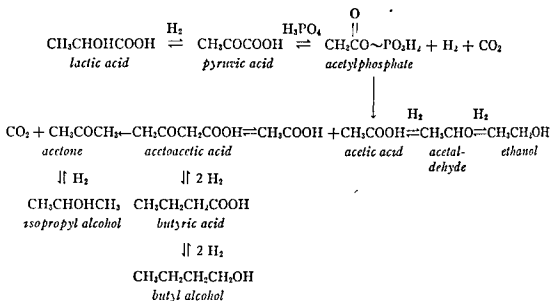
EPIZOOTIC ABORTION OF MARES

In addition to infection with *Salm. abortus-equi* or with streptococci, a form of epizootic abortion of mares has been described that appears to be caused by a virus. The disease is very contagious. Abortion occurs, after profuse sweating but no other clinical symptoms, at the 9th to 11th month. There is a slight vaginal discharge of short duration, with rapid and complete recovery of the mare. Dimock and Edwards (1936) observed several outbreaks in the United States. No bacterial cause could be found. In many of the aborted foetuses there was a clear yellow or reddish-yellow exudate in the pleural cavity, numerous small white foci of degeneration in the liver just beneath the capsule, congestion of the mesenteric and colonic lymph nodes, and petechial and lineal hæmorrhages in the heart. Pregnant mares, fed on fluids and tissues from infected foetuses, either before or after Seitz filtration, aborted in 18 to 34 days. Dimock and Edwards obtained doubtful results in their attempted transmission of the disease to guinea-pigs, but Miessner and Harms (1937) in Germany were apparently more successful. By subcutaneous and intraperitoneal inoculation of filtered material from the liver, spleen, kidneys, and stomach contents of aborted foetuses, they reproduced the disease in pregnant guinea-pigs, and carried on infection through 4 passages. The virus was grown in foetal cat lung (Randall *et al* 1954).

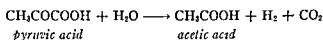
FOWL PLAGUE OR FOWL PEST

This is an acute infectious disease of fowls, and exceptionally of geese, which was studied by Landsteiner (1906) and Russ (1906). It does not appear to spread by contact, and the natural mode of infection is unknown. The incubation period is 3–5 days; the symptoms consist of depression and dullness, darkening of the comb and wattles, and inflammation of the buccal mucosa. The disease lasts 2–4 days, and is very frequently fatal (Hutyra and Marek 1926). The experimental disease may be produced in chickens by the inoculation of blood, nasal

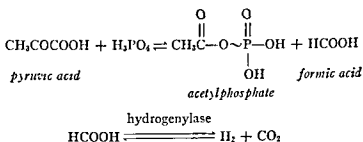
THE BUTYL ALCOHOL FERMENTATION



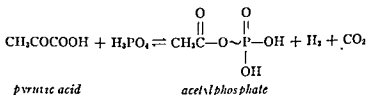
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Electronmicrography shows it to be irregularly globular in shape in the allantoic fluid and sperm-like in saline (Cunha *et al.* 1947, Bang 1948). The virus can be readily cultivated on the chorio-allantoic membrane of the developing chick embryo. It kills the embryo in 21-72 hours; *post mortem*, hæmorrhagic encephalitis and hæmorrhagic lesions, particularly in the developing feather follicles, are striking features (Burnet 1942). Inoculated by the allantoic route, it multiplies abundantly, and the allantoic fluid gives the Hirst agglutination reaction with chicken red blood corpuscles. After intranasal inoculation into mice, it produces consolidation of the lungs. In these and some other ways the Newcastle virus closely resembles the influenza virus of man. It is not, however, pathogenic for ferrets, nor does it appear to be antigenically related to the virus of influenza (Burnet 1942), though it behaves like it in many respects. It is found in the body fluids, organs, and excretions of affected birds. Cross-immunization tests show that the fowl plague and Newcastle viruses are distinct. The disease appears to have been introduced into Great Britain from time to time by infected carcasses from Central Europe.

The degree of infectivity of the Newcastle disease virus for *man* is in question. A few undoubted laboratory infections have occurred, usually characterized by conjunctivitis with mild constitutional symptoms. Nelson and his colleagues (1952) described an outbreak of conjunctivitis among workers engaged in eviscerating and canning poultry; the virus was isolated from the conjunctiva of 4 out of 10 patients examined. Howitt, Bishop, and Kissling (1948) ascribed to this virus a fairly widespread infection resembling influenza or non-paralytic poliomyelitis that occurred year after year in Alabama and Tennessee. The virus itself was not isolated, but neutralizing antibodies were found in the serum of the patients. Evans (1954) found that the serum of some patients convalescent from mumps or from glandular fever neutralized the Newcastle virus, though Newcastle antiserum did not neutralize the mumps virus. On infected farms some workers who had suffered from conjunctivitis contained neutralizing antibodies to the Newcastle virus in their serum to a significant titre.

Diagnosis of the disease in fowls is best made by inoculating the spleen into fertile eggs and testing the allantoic fluid. The disease is inhibited by specific antiserum. In controlling the disease the slaughter policy is recommended where practicable. Some success has been obtained with the use of formalized vaccines, vaccines prepared with crystal violet, and vaccines containing living attenuated virus, but no entirely satisfactory vaccine is yet available (see Doyle and Wright 1950).

INFECTIOUS LARYNGO-TRACHEITIS OF FOWLS

This disease has so far been reported mainly from North America and Australia, though cases have occurred in Great Britain (Asplin 1948). It appears to have been first recognized and studied in the United States by May and Tittsler (1925), Beach (1931), and others. Hyperacute, acute, chronic and asymptomatic forms are recognized clinically (Seddon and Hart 1935). Affected birds suffer from difficulty in respiration. The head is elevated, the neck extended, and air is drawn in through the half-opened beak with a loud wheezing sound. During expiration the head is lowered, and violent fits of coughing may occur, accompanied by the expulsion of masses of clotted blood and mucus. Fowls of all ages are susceptible. The incubation period is 7-12 days. The morbidity in an infected herd is 50-90 per cent. The case fatality varies in different outbreaks from 5-90 per cent.

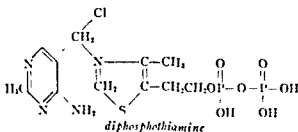
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⁴⁹ Barron and Friedemann, *Jour. Biol. Chem.*, 1941, 137 593.

egg passage, but a satisfactory vaccine is still awaited. The common method of protection is to infect the chicks with virulent material before egg production begins (Beaudette 1949).

BLUE COMB OF CHICKENS

SYNONYMS: Pullet disease, X Disease.

This disease has been observed in the north Atlantic and north central areas of the United States and on the Pacific coast. It affects pullets mainly 5-7 months of age, though chicks only 4 weeks old may be attacked. Turkeys may also suffer. The incidence is highest in the summer months. The morbidity and mortality in a flock are very variable. According to Jungherr and Levine (1941), who described the disease, the morbidity varies from 0.1 to 100 per cent. with a mean of about 20 per cent., and the case fatality from 0.2 to 53 per cent. with a mean of about 5 per cent. The disease is characterized by a fall in egg production and food consumption, darkening of the comb, droopiness, cyanosis, dehydration, diarrhoea or constipation, and occasionally pyrexia. Pathologically, the chief lesions are focal necrosis of the liver, serous petechiæ, enlargement of the pancreatic islands, patchy muscular degeneration, and nephrotic changes in the renal glomeruli and tubules. Jungherr and Levine regarded the disease as an endogenous toxæmia, but Waller (1942, 1944) brought evidence in favour of a virus origin. From the blood and liver of acutely affected birds he isolated a filtrable agent that grew readily on the chorio-allantoic membrane of the developing chick embryo. Susceptible chickens inoculated with infected membrane become depressed and cyanotic in 3-4 days. They do not die but, if killed after 4-5 days, they show subcutaneous œdema, generalized jaundice, hæmorrhages into the skeletal muscles, petechial hæmorrhages in other organs, congestion and swelling of the liver and kidneys, and acute catarrhal hæmorrhagic duodenitis. Fæces of experimentally infected birds are infective to other birds by the mouth. Intraperitoneal inoculation of susceptible chickens with a bacteria-free filtrate of infected fæces likewise reproduces the disease. The virus passes through a Chamberland L3 and a Sertz filter. Its relationship to the disease must await further inquiry. There is evidence that a vaccine prepared from the dried chorio-allantoic membrane of infected chick embryos may afford some protection (Waller 1944).

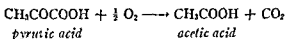
MISCELLANEOUS DISEASES OF FOWLS AND OTHER BIRDS

Eveleth, Bohn and Goldsby (see Bohn *et al.* 1949) described a disease of poults and baby chicks in North Dakota attended by a high mortality during the first 3 weeks of life. The outstanding lesion was necrosis of the abdominal wall around the navel. Bohn and his colleagues (1949) isolated a virus from poults dead of the disease and carried it by serial transfer through chick embryos. The disease was reproduced in chicks by feeding and by swabbing the navel with the amniotic fluid of dead embryos. The virus is more pathogenic for turkeys than for chicks.

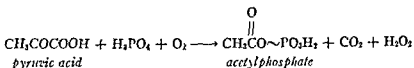
Levine and Fabricant (1950) described a highly fatal disease of young ducklings on Long Island in 1948. The principal findings at necropsy were an enlarged hæmorrhagic liver and swollen injected kidneys. A virus was isolated by inoculation into the allantoic cavity of 9-day-old chick embryos. It differed from the virus of Newcastle disease in its failure to agglutinate fowl red blood corpuscles, to kill embryos consistently within 48 hours, or to be neutralized by Newcastle antiserum. Virus from infected eggs repro-

The exact function of diphosphothiamine, or cocarboxylase, in the metabolism of pyruvic acid is not known, and the reversible oxidation-reduction of this coenzyme has not been demonstrated.

In *Bact. coli*⁵⁰ and the gonococcus,⁵¹ pyruvic acid is oxidized in a reaction in which diphosphothiamine, but not inorganic phosphate, is required, and electron transport involves the flavoproteins and the cytochrome system. In *Proteus vulgaris*⁵² the oxidation of pyruvic acid also proceeds without uptake of phosphate, but the pyruvic oxidase of this bacterium is a Mg-diphosphothiamine protein which apparently reacts directly with oxygen. In these types of pyruvic acid oxidation, the equation for the reaction is:



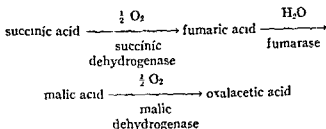
Lipmann⁵³ has described a third type of pyruvic acid oxidation in *Lactobacillus delbrueckii* in which the oxidation is coupled with the uptake of inorganic phosphate and acetylphosphate is formed.



The pyruvic oxidase of *L. delbrueckii* is a Mg-diphosphothiamine protein, and oxygen uptake is due to the presence of an autooxidizable protein (p. 71).

Anaerobic breakdown products of pyruvic acid are also oxidized by molecular oxygen. Ethanol is oxidized to acetic acid by the bacteria responsible for the vinegar "fermentation"; the propionic acid bacteria oxidize acetaldehyde to acetic acid, and acetic acid itself may be oxidized to CO_2 and H_2O .

Oxidation of the Four-Carbon Dicarboxylic Acids. Many bacteria rapidly oxidize the four-carbon dicarboxylic acids. Anaerobically, the reverse reactions occur, and oxalacetic acid may be reduced to succinic acid.



Szent-Gyorgyi and Krebs have shown that the oxidation of carbohydrates in higher animals is catalyzed by the four-carbon dicarboxylic acids, and such catalysts also occurs in bacteria. For example, in the oxidation of glucose by *Micrococcus lysodeikticus*, small amounts of fumaric acid cause an increased

⁵⁰ Sull. *Biochem. Jour.*, 1941, 35:380.

⁵¹ Barron and Lyman: *Jour. Biol. Chem.*, 1939, 127:143.

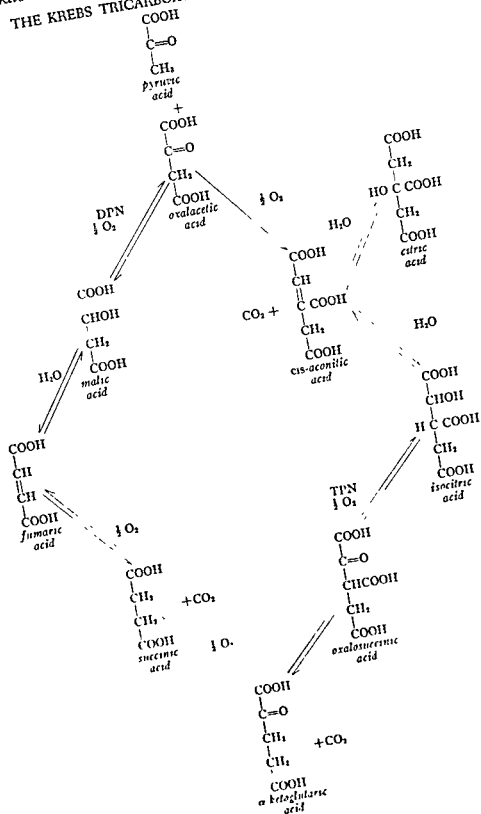
⁵² Stumpf. *Jour. Biol. Chem.* 1945, 159:529.

⁵³ Lipmann. Cold Spring Harbor Symposium on Quantitative Biology. 1939, 7:246. *Jour. Biol. Chem.*, 1944, 155:55.

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Aerobic Breakdown of Carbohydrate

THE KREBS TRICARBOXYLIC ACID CYCLE

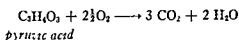


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oxygen uptake five times greater than the amount of oxygen required for the complete oxidation of the added fumaric acid to CO_2 and H_2O .⁵⁴

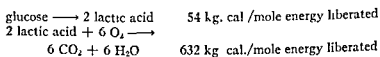
*The Szent-Gyorgyi Cycle.*⁵⁵ According to Szent-Gyorgyi such catalysis occurs because fumaric and oxalacetic acids act as hydrogen acceptors in the oxidation of glucose and are reduced to succinic acid which is reoxidized by molecular oxygen. This series of reactions is referred to as the Szent-Gyorgyi cycle. Many bacteria aerobically oxidize succinic acid faster than glucose itself, and the rate of anaerobic glucose oxidation in the presence of excess fumaric acid may be as great as the rate of aerobic glucose oxidation. Thus, it is possible for the Szent-Gyorgyi cycle to function in the respiration of some bacteria, but its actual occurrence has not been observed.

*The Krebs Cycle.*⁵⁵ A considerable portion of the carbohydrate metabolized by most aerobic organisms is oxidized completely to CO_2 and H_2O , usually by way of pyruvic acid as an intermediate. In a wide variety of organisms, the complete oxidation of pyruvic acid is brought about, not by a single reaction, but by a cyclic series of reactions, the tricarboxylic acid cycle of Krebs. In this scheme, pyruvic acid condenses with oxalacetic acid to form a six-carbon tricarboxylic acid which is oxidized and decarboxylated in a series of reactions ending in the regeneration of oxalacetic acid. The net reaction of the cycle is:



The tricarboxylic acid cycle has been demonstrated in mammalian and avian tissues and in protozoa. It probably also functions in plants and fungi. While almost all of the reactions of the tricarboxylic acid cycle occur in bacteria, it has not been shown that the cycle functions in the oxidation of pyruvic acid by bacteria. The chief difficulty in demonstrating the Krebs cycle in bacteria lies in the inability of most bacteria, *Bact. coli* for example, to oxidize citric, isocitric or *cis*-aconitic acid. Those bacteria which can oxidize the tricarboxylic acids (*Bact. aerogenes*, etc.) apparently form only succinic acid, acetic acid and CO_2 , not α -ketoglutaric acid as required by the tricarboxylic acid cycle. However, Werkman⁵⁴ believes that some sort of a tricarboxylic acid cycle may be operative in bacteria.

Energy Yield from Aerobic Oxidation of Carbohydrates. The yield of useful energy from complete aerobic oxidation of glucose to CO_2 and H_2O is much greater than the yield from anaerobic conversion of glucose to lactic acid, ethanol, etc.



In the tissues of higher animals, glucose and other carbohydrates are oxidized

⁵⁴ Werkman in *A Symposium on Respiratory Enzymes*, University of Wisconsin Press, Madison, 1942, p. 258.

⁵⁵ For evaluation of the role of the Szent Gyorgyi cycle and the tricarboxylic acid cycle in bacterial metabolism, see Krebs *Advances in Enzymology*, 1943, 3 191, and reference 54.

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Synthesis of Cell Substances from Carbohydrates

almost completely to CO_2 and H_2O under aerobic conditions. In these tissues, the energy liberated in the oxidation of carbohydrate is conserved with great efficiency in high energy phosphate bonds. As many as fifteen such bonds may be generated by the oxidation of a single mole of pyruvic acid.⁷⁶ This represents about the same energetic efficiency as obtained in the anaerobic oxidation of glucose. When bacteria oxidize carbohydrate completely to CO_2 and H_2O , it is probable that a similar formation of high energy phosphate bonds occurs, but it has not yet been experimentally demonstrated. When aerobic oxidation of carbohydrate is incomplete, as in many bacterial oxidations, the energy change is not nearly so great. For instance, the utilizable energy released in the oxidation of pyruvic acid to acetic acid and CO_2 is only sufficient to generate one energy-rich phosphate bond.

Synthesis of Cell Substances from Carbohydrates. The breakdown of carbohydrates by the series of energy-yielding reactions just discussed is actually only one of several ways in which carbohydrates are utilized by the bacterial cell. They may also be combined with CO_2 to form new substances, converted into oligosaccharides and polysaccharides, or transformed into non-carbohydrate cell substances. These are the processes by which bacteria change the carbohydrates of the culture medium into their own highly specific cellular components. Such reactions generally do not proceed spontaneously because they require an external source of energy. The energy-requiring actions may be designated as synthetic reactions in contrast to the energy-yielding degradative reactions. In the living cell, the two types of reactions occur in such a manner that the energy released in the breakdown of carbohydrates is used in part for the synthesis of other carbohydrates, usually in the form of high energy phosphate bonds.

Assimilation of Carbon Dioxide.⁷⁷ It has been known for many years that CO_2 is involved in the metabolism of heterotrophic bacteria, because it had been repeatedly observed that many bacteria did not grow when all CO_2 was removed from the culture medium with CO_2 -free air. However, proper interpretation of these findings did not come until 1935, when Wood and Werkman discovered that the heterotrophic propionic acid bacteria fix carbon dioxide into organic linkage, mainly in the carboxyl groups of the four-carbon dicarboxylic acids. Later investigations employing isotopically labelled CO_2 confirmed this original observation and demonstrated the occurrence of CO_2 fixation in other bacteria, yeasts, fungi, protozoa and higher animals.

Several different primary fixation reactions are responsible for the assimilation of CO_2 in heterotrophic bacteria. They may be classified on the basis of the substance which reacts with CO_2 (the specified organism is that in which the reaction was first reported):

- (1) No C-C linkage
in *Methanobacterium omelianskii*
 $\text{CO}_2 + 4 \text{ Acceptor H}_2 \longrightarrow \text{CH}_4 + 4 \text{ Acceptor} + 2 \text{ H}_2\text{O}$

⁷⁶ Ochoa Jour. Biol. Chem., 1943, 151-493.

⁷⁷ This subject has been reviewed by Werkman and Wood. Advances in Enzymology, 1942, 2 135, Krebs Ann. Rev. Biochem., 1943, 12 529, and Wood Physiol. Rev., 1946, 26 198.

CHAPTER 89

FILTRABLE VIRUS DISEASES—*Continued*

E. GROUP CHARACTERIZED BY TUMOUR FORMATION

THE group of virus diseases characterized by tumour formation is of particular interest in affording a possible clue to the genesis of neoplasms. In this chapter we must confine ourselves to factual description; but those who are interested in the wider aspects of the subject would do well to refer to the far-reaching speculations of Andrewes (1939*b*, 1950), of Rous (1943), and of Gye (1949). It should be pointed out that there is no sharp line of demarcation between viruses producing tumours and other viruses. Some viruses cause proliferation of the cells they attack, others necrosis and destruction. Some, such as that of rabbit fibroma, have variants, one causing proliferation, the other necrosis and inflammation (Andrewes 1951). Nevertheless there are groups of viruses having strong tendencies to cause proliferation, and with these we shall deal here.

FOWL LEUCÆMIA

This is an infectious disease of the blood-forming organs, associated with atrophy of the bone marrow and changes in the viscera. The disease may be transmitted to normal fowls, but not to other birds, by intravenous inoculation with the citrated blood of infected fowls. According to Ellermann (1921), the virus can pass through a Berkefeld candle. By electronmicrography it appears to be about 120 $m\mu$ in diameter (Sharp *et al.* 1952). Ellermann found that one strain of virus gave rise to three different types of leucæmia: (1) a myeloid type; (2) a lymphatic type; (3) an intravascular lymphoid type, characterized by intravascular deposits of lymphoid cells. An increase of virulence was noted during its passage through fowls, shown by a shortening of the interval between inoculation and death, from 15 to 20 weeks at the commencement, to 6 to 8 weeks at the end of the series of experiments. In spite, however, of the increased rate of killing, the infecting power of the virus, as judged by the proportion of successful inoculations, remained approximately constant at 20–40 per cent. Ellermann was unable to produce active immunity to the disease by subcutaneous inoculation of virulent material.

Furth (1932) found that infection could be transmitted by 0.000,001 ml. of cell-free plasma, and that the virus could withstand drying for at least 54 days. He also (1933) described a new transmissible strain of chicken leucosis which gave rise to (1) lymphomatosis with or without tumour formation, (2) myelocytomatosis with or without leucæmia, (3) endothelioma. The histology of the disease is beautifully illustrated in the monograph by Andersen and Bang (1928).

- (2) C_1 - C_1 addition
in *Cl. acidurici*
 $2\text{CO}_2 + 4\text{ Acceptor-H}_2 \longrightarrow 2\text{CH}_3\text{COOH} + 4\text{ Acceptor} + 2\text{H}_2\text{O}$
- (3) C_2 - C_1 addition
 a. in *Cl. butylicum*
 $\text{CH}_3\text{COPO}_3\text{H}_2 + \text{H}_2 + \text{CO}_2 \rightleftharpoons \text{CH}_3\text{COCOOH} + \text{H}_2\text{PO}_4$
 b. in *Bact. coli*
 $\text{H}_2 + \text{CO}_2 \rightleftharpoons \text{HCOOH}$
 $\text{HCOOH} + \text{CH}_3\text{COPO}_3\text{H}_2 \rightleftharpoons \text{CH}_3\text{COCOOH} + \text{H}_2\text{PO}_4$
- (4) C_2 - C_1 addition
 in *Propionibacterium pentosaceum*

$$\begin{array}{ccc} \text{COOH} & & \text{COOH} \\ | & & | \\ \text{C=O} + \text{CO}_2 & \rightleftharpoons & \text{C=O} \\ | & & | \\ \text{CH}_3 & & \text{CH}_2 \\ & & | \\ & & \text{COOH} \end{array}$$

Of these modes of addition, the carboxylation of pyruvic acid to oxalacetic acid, the Wood-Werkman reaction, is the most widely distributed. An additional primary fixation reaction, the addition of CO_2 to α -ketoglutaric acid to form oxalosuccinic acid, occurs in animal tissues but has not yet been found in bacteria.⁵⁴ Reductive fixation of CO_2 (fixation reactions 1 and 2) is accompanied by the oxidation of a hydrogen donor, which may be any of several simple organic compounds, such as ethanol. In non-reductive fixation (fixation reactions 3 and 4) the CO_2 appears in the carboxyl group of an organic acid without being reduced. After CO_2 has been incorporated into an organic compound by one of the primary fixation reactions, further metabolic reactions may lead to the distribution of the fixed CO_2 among many different substances. Thus, Wood and Werkman found that the CO_2 fixed in oxalacetic acid by the propionic acid bacteria also appeared in the other four-carbon dicarboxylic acids and in propionic acid.

The ability to fix CO_2 into organic linkage was once thought to be the exclusive property of autotrophic organisms, but after demonstration of CO_2 fixation in the heterotrophic propionic acid bacteria, the line between autotrophes and heterotrophes was redrawn by assuming that only autotrophes can join two molecules of CO_2 together in organic linkage. However, when it was found that *Cl. acidurici* can synthesize acetic acid from two molecules of CO_2 , even this difference between the two metabolic types was destroyed. The distinction between autotrophic and heterotrophic CO_2 fixation seems to be only quantitative: heterotrophes derive only a fraction of their carbon compounds from CO_2 , while autotrophes obtain all of their compounds by assimilation of CO_2 .

Studies of the energetics of CO_2 assimilation have emphasized the close similarity of autotrophic and heterotrophic mechanisms. Incorporation of CO_2 into an organic compound requires the expenditure of energy. In the autotrophe, *Thiobacillus thiooxidans*, CO_2 is fixed with energy gained from the oxidation of sulfur to sulfate, and ATP takes part in the transfer of energy from the sulfur-oxidizing to the CO_2 -fixing system (p. 81). In a comparable

⁵⁵ Ochoa. Jour. Biol. Chem., 1948, 174 133.

KCl, and a fragment of chick embryo have been added. He believed that the tumour-forming activity of this virus is dependent on the presence of another, non-living factor, which determines the type of cell which is infected, and hence the type of tumour which is produced. This second, auxiliary agent he called the "specific factor." The evidence in favour of the dual nature of the active agent in tumour filtrates consists in the differential action of aerobic incubation and of various chemical agents. In an extensive series of experiments Gye found that (a) primary "cultures" incubated aerobically at 37° C. for 3 days were inactive; (b) filtrates, obtained by passing the ground-up tumour through a mixture of paper pulp and sand, when treated with chloroform, were inactive; but that (c) the inactivated "cultures" and the chloroform-inactivated filtrates, when acting in combination, gave rise to typical tumours. He would regard the specific factor in the "cultures" as being destroyed by aerobic incubation, and the virus in the tumour filtrate as being destroyed by the chloroform. He would also extend the conclusions based on these results to malignant neoplasms in general. Gye's conclusions have never been satisfactorily confirmed, though they have stimulated considerable interest in the virus causation of cancer (see also Gye and Mueller 1929, Amies and Carr 1939). According to Epstein (1951) repeated freezing and thawing destroys the Rous sarcoma cells but not the virus itself. Similar experiments to study the effect of freezing, drying and thawing on tumours of different types with a view to separating a possible virus from the cells in which it is contained are in progress (see Craigie 1949). The observations of Duran-Reynals (1942) that the Rous virus, when adapted to ducklings, produces a tumour with a different histological pattern, have likewise aroused interest by indicating the existence of a plasticity in the virus that would not otherwise have been suspected.

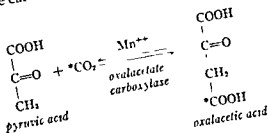
Besides the Rous sarcoma, other filtrable tumours of fowls are known. McIntosh (1933) and McIntosh and Selbie (1939) reported that tumours induced by tar may be transmitted to normal birds by cell-free filtrates; and Selbie and McIntosh (1939) found that the filtrability of fowl tumours may be increased by such procedures as proteolytic digestion or freezing and thawing, which presumably act by liberation of virus from the cells in which it is growing. The demonstration by Ledingham and Gye (1935) of what appear to be elementary bodies in infective filtrates, and the immunological studies of Andrewes (1931, 1932, 1933, 1936b, 1939a), suggest more and more strongly that viruses, not differing in any important respect from those known to give rise to ordinary infectious diseases, play an essential part in the causation of these tumours (see Andrewes 1934). The particle size of the Rous sarcoma virus was determined by Elford and Andrewes (1935, 1936). By the gradocol membrane technique it appears to be 75-150 m μ in diameter, and by the centrifugation method 60-70 m μ .

MOLLUSCUM CONTAGIOSUM

This is a contagious skin disease of man characterized by the formation of small red masses, which later take on a warty appearance, undergo necrosis, and discharge caseous material. MacCallum (1892) described peculiar bodies in material taken from the disease, which he regarded as due to nucleolar extrusion. They were later described by Lipschütz (1907) as very small rounded bodies, about 0.25 μ in diameter, arranged singly or less often in pairs. They occur in the epidermal cells, are intracytoplasmic, and stain a brilliant red with eosin or phloxine. These molluscum bodies were at one time considered to be parasites, but are now generally

Synthesis of Cell Substances from Carbohydrates

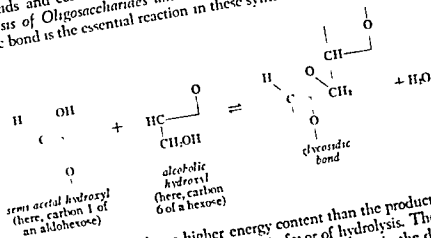
in fashion, energy for the reductive fixation of CO_2 by *Methanobacterium omelianskii* and *Cl. acidurici* is obtained from the simultaneous oxidation of organic substrates. In the carboxylation of acetylphosphate to pyruvic acid in *Bact. coli* and *Cl. butylicum*, the high energy phosphate bond of acetylphosphate is probably the source of energy used in fixation, since pyruvic acid is not formed when acetylphosphate is replaced by acetic acid. Carboxylation of pyruvic acid to oxalacetic acid in the Wood-Werkman reaction is catalyzed by the enzyme oxalacetate carboxylase, which has been found both in bacteria and in animal tissues.



The energy change in the reaction is such that the equilibrium of the reaction is far to the left in favor of decarboxylation of oxalacetic acid. However, when ATP is added to pyruvic acid and CO_2 in the presence of Mn^{++} and oxalacetate carboxylase, oxalacetic acid is formed in amounts detectable by isotopic tracer techniques. The function of ATP in this reaction is unknown.

Assimilation of CO_2 in autotrophs is the source of all organic compounds synthesized by these organisms, but in heterotrophs the function of CO_2 fixation is not so clear. However, from the relatively few direct products of CO_2 fixation, an almost unlimited number of compounds may be produced in further metabolic reactions, and it has been suggested that CO_2 fixation functions in the synthesis of essential metabolites, notably the four-carbon dicarboxylic acids and certain amino acids.

Synthesis of Oligosaccharides and Polysaccharides. The formation of the glycosidic bond is the essential reaction in these syntheses.



Since the glycosidic bond has a higher energy content than the products of its hydrolysis, the equilibrium of the reaction is in favor of hydrolysis. Therefore, an external source of energy must be used to drive the reaction in the direction of synthesis of the glycosidic bond. There are at least two mechanisms for this.

round or rod-shaped bodies (see also Lipschütz 1927). The pathogenesis of the disease is described by Fenner and Woodroffe (1953).

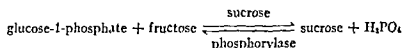
The virus is widely distributed throughout the tissues, though most abundant in the swollen tissues and lymph glands. It is killed by heat at 50° C. in 15 minutes. It is quickly inactivated by a pH of less than 4.6. It withstands the action of glycerol for a year in the ice-chest. It is highly species-specific, and appears to be almost uniformly fatal for the domestic rabbit (see Rivers 1930, Findlay 1933, Hyde and Gardner 1933, Ledingham 1937). Elementary body suspensions were prepared by Rivers and Ward (1937). They were shown to contain a soluble antigen, which could be demonstrated as a precipitinogen in the serum of infected animals. This antigen, which is a heat-labile protein, stimulates the production in rabbits of precipitins and agglutinins, but not of neutralizing antibodies (Rivers *et al.* 1939). By microscopical examination van Rooyen (1937*b*) estimated the elementary bodies to be about 0.31–0.36 μ in diameter, but other methods suggest a size of only 125–225 $m\mu$ (see Mueller 1941, Stanley 1943). Electronmicrographs reveal elementary bodies, mainly 100–400 $m\mu$ in diameter, within the stellate cells, though smaller particles about 30–45 $m\mu$ across may also be seen (Epstein *et al.* 1952). The virus can be cultivated on the chorio-allantoic membrane of the developing chick embryo (Lush 1937, Hoffstadt *et al.* 1941). There are reasons for regarding the virus as belonging to the pox group. Both vaccinia and myxoma viruses have the same brick-shaped appearance with dense pepsin-resistant central bodies, and both are of the same size (Farrant and Fenner 1953).

Aragão (1942, 1943) first showed that the reservoir of myxomatosis in Brazil was the native rabbit, and that infection was transmitted by mosquitoes. The disease was introduced into Australia in 1950 in the hope of exterminating the enormous population of wild rabbits. After a long latent period it flared up and caused a most destructive epizootic. In the summer spread occurred mainly along rivers and on flooded lands where mosquitoes breed (Ratcliffe *et al.* 1952). According to Araújo (1942, 1943) the mosquito acts purely as a mechanical carrier. After insertion into infected epithelial cells, the proboscis may remain infective for 17 days or even longer (see Fenner and Woodroffe 1952). The disease was introduced into France in 1952 and rapidly spread over the whole country and on to other parts of Europe including Great Britain. Transmission of infection over long distances and during the winter as well as the summer months indicated that the mosquito alone was not responsible; it is thought that birds (Ramon 1953) and rabbit fleas (*Spilopsyllus cuniculi*) may have played a part (Andrewes 1954). Whether the disease will prove to be of lasting value for combating the rabbit, or whether the rabbit will develop a resistance towards it as the Bombay rats have done towards plague, the future alone will tell.

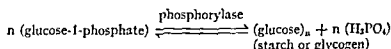
The relation of this virus to the virus of infectious fibromatosis of rabbits will be discussed below. A neurotropic strain of the myxoma virus was developed by Hurst (1937) as the result of serial passage through the brain of rabbits. This strain did not prove fatal on intracutaneous inoculation, and the histological picture to which it gave rise differed from that of the parent strain. Animals inoculated with the neuromyxoma strain were protected against subsequent inoculation with the myxoma-producing virus. Vaccination, however, in practice is generally carried out with a strain of the fibroma virus, and is used particularly for the protection of hutch rabbits in countries where the disease is endemic. Though the fibroma virus protects against the clinical manifestations of myxomatosis, it apparently does

nisms for synthesis of polysaccharides and oligosaccharides, both of which meet the energy requirement with the high energy phosphate bonds of ATP.

If the semi-acetal hydroxyl is converted into a phosphate ester, glucose-1-phosphate, the glycosidic bond is easily formed because the phosphate ester bond has about the same energy content as the glycosidic bond itself. Thus, sucrose may be formed in *Pseudomonas saccharophila* and *Leuconostoc mesenteroides* by the reaction.³⁰:



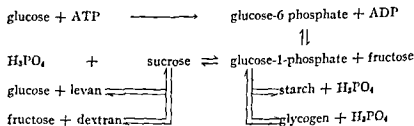
Cori³⁹ has carried out the *in vitro* synthesis of starch and glycogen by similar reactions catalyzed by phosphorylases from plant and animal sources.



A small amount of preformed polysaccharide is necessary for this reaction, because phosphorylase acts to add new glucose units to existing terminal units in the polysaccharide. Although synthesis of polysaccharides by this method has not been observed in bacteria, it is likely that it does occur.

New glycosidic bonds may also be formed at the expense of existing glycosidic linkages. Such reactions occur with little energy change and are thus reversible. The polymeric degradation of sucrose to form levans and dextrans has already been described. This mode of synthesis is widely distributed in bacteria. Dextran, a polymer of glucose units joined in 1,6-glycosidic linkages, is found in *Leuconostoc mesenteroides* and in some types of pneumococcus. Levans are polymeric 2,6-glycosides of fructose, and levans of close chemical and immunological similarity are present in several species of *Bacillus*, one of *Aerobacter*, and two of *Streptococcus*.³¹

The manner in which the energy for all these syntheses is eventually derived from ATP may be illustrated in a diagram adapted from Avineri-Shapiro and Hestrin⁶⁰:



Oxidative Assimilation.⁶¹ In a culture of growing bacteria it is obvious that the constituents of the medium are being converted into bacterial cell material. However, it was not fully realized before the work of Barker and Giesberger that even resting, non-proliferating cells do not oxidize their carbohydrate substrates completely to CO_2 and H_2O but instead assimilate a large portion of

³⁰ Cori, Swanson, and Cori. *Fed. Proc.*, 1945, 4 234.

⁶⁰ Avineri-Shapiro and Hestrin. *Biochem. Jour.*, 1945, 39 167.

⁶¹ Clifton. *Advances in Enzymology*, 1946, 6:269.

moments after injection. Proceeding on this assumption, they attempted to measure the distribution of antibody between blood and tissue by comparing the concentration of antitoxin which neutralizes toxin on direct injection of the two into the tissue, with the blood concentration required to protect the tissue against an injection of toxin alone (indirect neutralization). In guinea-pigs the ratio of the two concentrations of antitoxin was about 20 for diphtheria and staphylococcal toxins given intradermally; in guinea-pigs and rabbits it varied between 50 and 1 for different batches of tetanus toxin given intracerebrally (Friedemann *et al.* 1939, Friedemann and Zuger 1939*b*; see also Friedemann *et al.* 1944, 1946*a, b*). Other workers have found no evidence of a rapidly established distribution. Andersen (1937), for example, observed that the antibody content of the skin of rabbits passively immunized against vaccinia by the intravenous route was relatively low after one day, and was equal to that of the blood after 7 days. Miles (1949), however, demonstrated that "indirect" neutralization of intradermal diphtheria toxin by circulating antitoxin was not immediate, but progressive, depending presumably on the rate of passage of antibody through the capillary endothelium; and that toxin was fixed to the tissues at a relatively slow rate. He concluded that, since the indirect neutralizing dose was in part determined by these two processes, its ratio to the direct neutralizing dose was not a valid measure of the intra- and extra-vascular concentrations of antibody.

Friedemann's ratios, though they are not measures of the distribution of antibody between blood and tissues in the uninfected animal, are nevertheless indications of the magnitude of the antibody levels required in the blood of passively immunized animals for a protective effect in the tissues, irrespective of whether that protection is afforded by antitoxin already in the tissues, or by antitoxin passing from the blood to the tissues. When we consider the greater complexity of factors concerned in the action of tissue cells and fluids on bacteria, it will be clear that the blood levels necessary for the suppression of a tissue infection may be much higher (see also Fox 1943, Schlesinger, Olitsky and Morgan 1944).

The Availability of Antibody at Mucous Surfaces.

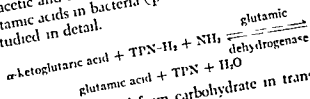
Antibody is demonstrable in the contents of the alimentary canal ("copro-antibody"), in the urine, and in the uterine cavity. Although certain serum globulins have been shown to pass from the circulation to the lumen of the gut, antibodies in the circulation by no means always appear in the faeces. Cholera antibody in the guinea-pig, for example (see Burrows *et al.* 1947, Burrows and Havens 1948, Koshland and Burrows 1950), appears to be formed locally in response to a local antigenic stimulus, and is moreover protective. Local formation appears also to be responsible for the presence of dysentery copro-agglutinins in man and rodents (González and Koppisch 1951), and of antibodies to *Trichomonas fetus* in the uterine cavity of the cow (Kerr and Robertson 1947; see also Pierce 1947, 1953). Naylor and Caldwell (1953) distinguish the local formation of salmonella agglutinins in the human bladder, as a sequel to infection, from the appearance of circulating antibodies in the urine, as the result of unrelated pathological changes in the bladder wall leading to exudation of serum proteins. In experimental *V. cholerae* gastro-enteritis of guinea pigs, which is mainly a superficial infection of the alimentary mucous membranes, Burrows and Ware (1953) demonstrated free antibody in the lumen of the gut of parenterally immunized animals associated with an immunity to oral infection with the vibrio. They also passively immunized guinea-pigs by antibody given orally.

The observation of MacLeod and his colleagues (1945) that immunization of soldiers with certain type-specific pneumococcal polysaccharides significantly diminished the carrier rate for the corresponding pneumococcal types, but left unaffected the carrier rate for unrelated types, is also consistent with the view that antibody

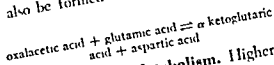
Regulation of Carbohydrate Metabolism

the carbohydrate into cell substance. In the colorless alga, *Prototheca zopfii*, the first organism studied by Barker, 50 to 80 per cent of simple substrates such as acetic acid and ethanol were assimilated into cell substances with the empirical formula of carbohydrate. This phenomenon, called by Barker oxidative assimilation, is of general occurrence in microorganisms and has been observed in several species of bacteria, yeasts and algae. In several experiments, the assimilated material has actually been identified as carbohydrate. Low concentrations of certain cell poisons, sodium azide and 2,4-dinitrophenol in particular, may inhibit the oxidative assimilation of a substrate and allow its complete oxidation to take place, apparently without inhibiting other metabolic processes. It is of interest that both these poisons interfere with the formation of energy-rich phosphate bonds.⁶² However, there is as yet no direct evidence that energy-rich phosphate bonds are involved in oxidative assimilation. Clifton has suggested that oxidative assimilation is brought about, not by energetic coupling of synthetic and degradative reactions, but by oxidative degradation of carbohydrate to simple "building blocks" such as acetaldehyde which may be converted into carbohydrate through a series of reactions not requiring energy from an independent system.

Formation of Amino Acids from Carbohydrates. The carbon skeleton of metabolized carbohydrate may be converted into amino acids, purines, pyrimidines, fatty acids, etc. The ability of heterotrophic bacteria to carry out these transformations is well illustrated by the growth of organisms such as *Bact. coli* in a medium containing glucose as the sole organic compound. Although other carbohydrates and carbohydrate derivatives undoubtedly form amino acids, the four-carbon dicarboxylic acids appear to be especially active in amino acid formation. Oxalacetic and α -ketoglutaric acids may be reductively aminated to aspartic and glutamic acids in bacteria (p. 109), and the formation of glutamic acid has been studied in detail.



Amino acids may also be formed from carbohydrate in transamination reactions



Regulation of Carbohydrate Metabolism. Higher organisms possess complex regulatory mechanisms which determine the pathways and control the rate of carbohydrate metabolism. These mechanisms are largely hormonal in nature. Bacteria possess no such formidable regulatory mechanisms, but even in bacteria the metabolism of carbohydrate is governed by the environment in which the cells are suspended. The temperature, the pH of the medium, the concentration of available substrate, and the presence or absence of oxygen may affect the rate of metabolism and the nature of the end products produced. A classical example of such a controlling factor is the effect of molecular oxygen upon the metabolism of organisms with both aerobic and anaerobic

⁶² For literature citations, see Lardy and Elvehjem, *Ann. Rev. Biochem.*, 1945, 14-16.

has been adapted to serial passage through the domestic rabbit loses this property after a single passage through the cotton-tail rabbit (Selbie *et al.* 1948). The interesting observation was made by Syverton and Berry (1947) that papilloma cells could be infected with certain other viruses, such as B virus, myxoma virus and vaccinia virus. The papilloma virus can infect epidermal tumours induced by tar, and may bring about in them an abrupt change into carcinomata; direct demonstration of the virus in the carcinomatous tumour has so far failed, but its presence can be shown by serological methods (Rous 1943). An even more rapid method of bringing about the carcinomatous state is to apply methylcholanthrene and tar to virus-induced papillomata; the chemical agents appear to act, not as ordinary stimulants of cell proliferation, but as specific carcinogens affecting the virus (Rous and Friedewald 1944).

The papilloma virus is filtrable through a Berkefeld candle, resists the action of glycerol, and is killed by exposure to 70° C. but not 67° C. for 30 minutes. By the gradocol membrane technique its size has been computed as 23-35 $m\mu$, and by the centrifugation technique as 32-50 $m\mu$ (Schlesinger and Andrewes 1937). Studies by the electron microscope suggest that the particles are spherical and have a mean diameter of about 44 $m\mu$ (Sharp *et al.* 1942). Rabbits with experimentally produced papillomata are partly or completely immune to re-infection, and their serum has neutralizing and complement-fixing properties (see Kidd 1938). Artificial immunization by intraperitoneal injection of glycerolated or non-infective virus has been described (Shope 1937).

ORAL PAPILLOMATOSIS OF RABBITS

Parsons and Kidd (1913) met with a disease among domestic rabbits in New York characterized by the occurrence of small, usually multiple, benign papillomata on the under side of the tongue. They persist often for several months but, unlike the Shope papilloma, do not become malignant. The disease can be reproduced in rabbits and horses, but not in other animals, by local inoculation of a tumour suspension. The resulting lesions are strictly confined to the oral mucosa—a site which is not affected by the Shope virus. Infection does not spread readily by contact. It is probably transferred from the mother to the young by suckling, and remains latent till it is activated by trauma or, experimentally, by tar. The virus passes through a Berkefeld N candle. In water-clear suspensions it is destroyed by exposure to 75° C. but not 65° C. for 30 minutes. In 50 per cent. glycerol Locke solution in the ice-chest it survives for 1-2 years at least.

HODGKIN'S DISEASE (LYMPHADENOMA)

Little is known of the aetiology of this fatal disease, in which characteristic histological changes are usually found in the enlarged lymph glands and lymphoid deposits. Gordon (1933, 1934, 1936) described the reproduction of meningo-encephalitis in rabbits by the intracerebral inoculation of autolysed gland suspensions. Elementary bodies are said to be distinguishable in human material and in impression preparations of infected rabbit's brain. Gordon's test is said to be of diagnostic value in about 75 per cent. of cases of Hodgkin's disease (van Rooyen 1934, 1937a). The encephalitogenic agent is said to pass through British Berkefeld and Seitz EK filters; to be inactivated by exposure to a temperature of 80° C. for 30 minutes; to withstand 0.5 per cent. phenol at 37° C. for probably a week

mechanisms for carbohydrate metabolism—that is, the facultative anaerobes. Early in his studies on the fermentative activities of microorganisms, Pasteur observed that the anaerobic fermentative breakdown of carbohydrate is lessened in the presence of oxygen. Many years later, Warburg called this phenomenon the Pasteur effect or Pasteur reaction.⁶³ The Pasteur effect has been studied extensively by Meyerhof, Warburg, Lipmann and many others, who have found that oxygen inhibits fermentation in almost all facultatively anaerobic cells, whether they are bacteria, yeasts, or the cells of higher plants and animals. The value to a facultative anaerobe of such a regulatory mechanism is obvious. By means of the Pasteur effect, the anaerobic fermentative breakdown of carbohydrate is blocked in the presence of oxygen, and energy is furnished by the far more efficient aerobic oxidation of carbohydrate. However, the actual mechanism of the Pasteur effect is far from obvious and is still the subject of spirited controversy. At present, it is generally believed that anaerobic fermentation is not inhibited by aerobic respiration but by the direct action of molecular oxygen upon some portion of the anaerobic breakdown mechanism.

NITROGEN METABOLISM⁶⁴

The metabolism of nitrogenous compounds by bacteria is less well understood than that of the carbohydrates, and the decomposition of these compounds is somewhat more complex in that the ring structures of the aromatic amino acids make their breakdown a series of special cases. In general, the decomposition of proteins and amino acids is of less quantitative importance in the processes of respiration except in certain cases such as that of the obligate anaerobes. The metabolism of these compounds is of no less importance, however, and the significance of the mechanisms of synthesis, which are intertwined with those of carbohydrate synthesis, is becoming increasingly clear, not only in relation to particular questions such as that of the mechanism of action of the chemotherapeutic drugs, but also in that the microorganisms have certain unique advantages in the investigation of the general biological problems of anabolism.

The Hydrolysis of Proteins. The ability to make use of amino acids is widespread among the bacteria, with the formation of compounds such as hydrogen sulfide, indol, amines and the like which are associated with the decomposition of proteins. The ability to hydrolyze native proteins to their amino acid constituents is, however, one that is possessed by relatively few kinds of bacteria. The limited occurrence of this capability is sufficiently marked that it is customary to speak of bacteria as fermentative or proteolytic in type according to whether carbohydrate oxidation or proteolysis characterizes their biochemical activities.

As compared with knowledge of the proteolytic enzymes of higher organisms, very little is known of the bacterial proteases.⁶⁵ In general they appear

⁶³ See the reviews by Burk, *Cold Spring Harbor Symposium on Quantitative Biology*, 1939, 7:461, and Lipmann, in *A Symposium on Respiratory Enzymes*, University of Wisconsin Press, Madison, 1942, p. 48.

⁶⁴ See the review by Gale, *Ann. Rev. Microbiol.*, 1947, 1:141.

⁶⁵ See Haines, *Biol. Rev.*, 1934, 9:235.

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The Hydrolysis of Proteins

to be tryptic in nature, i.e., they act in slightly alkaline media, whereas in protozoa and probably in fungi, pepsin-like enzymes occur. It is probable that a number of proteolytic enzymes are possessed by bacteria analogous to those occurring in higher organisms. Available evidence indicates, for example, that decomposition may be carried to the polypeptide stage by one or more enzymes, while further dissolution is accomplished by means of another enzyme or group of enzymes.

The bacterial proteases are, for the most part, extracellular enzymes which are diffused into the medium and are present in sterile filtrates of cultures. Presumably protein molecules are too large to diffuse into the bacterial cell and are partially hydrolyzed outside. Split products probably diffuse into the cell to be further decomposed. Intracellular bacterial proteases have, however, been found⁶⁶

Of the bacterial enzymes the proteases appear to be the most markedly affected by the medium upon which the organisms producing them are grown. Chief among the factors influencing their production is the nitrogenous material present in the medium, in general, highly active filtrates may be obtained only from cultures in protein-containing media, although the detection of proteolytic activity in cultures grown in some of the so-called "synthetic" media containing no protein material indicates that the presence of substrate is not essential to the formation of the enzymes. The inorganic salts present are also of importance. It has been shown, for example, that the presence of magnesium and calcium salts is necessary for the production of gelatinase, the former stimulating growth but the latter actively stimulating the production of the enzyme. Undoubtedly a series of other environmental factors affect the production of proteolytic enzyme, but present knowledge is extremely fragmentary.

The ability to liquefy gelatin, a character of considerable value in the biochemical differentiation of bacteria, is often taken as indicative of the proteolytic potentialities of these organisms. Many organisms, however, which liquefy gelatin are unable to break it down further to amino acid units which may be used. *Staphylococcus aureus*, for example, although liquefying gelatin, is unable to hydrolyze the protein to abiuret substances and, presumably, can neither assimilate nor oxidize this substance. The digestion of gelatin by actively proteolytic organisms such as *Proteus vulgaris* is, on the other hand, carried rapidly to the amino acid stage, and evidences of the further decomposition of the amino acids, such as the liberation of ammonia, soon appear.

The resistance of pure native proteins, coagulated proteins and even purified protease to attack by actively proteolytic bacteria in the absence of other food materials is well established. Such bacteria, inoculated into a mineral salt solution containing a pure protein such as crystalline egg albumin, do not multiply but in time die off. If, however, a small amount of peptone or meat extract is added to such a medium, the bacteria grow well and decompose the pure protein in a normal way. It is probable that extracellular protease inoculated with the bacterial cells is thereby so diluted that no hydrolysis takes place and, in the absence of smaller molecules that can diffuse into the cells

⁶⁶ Allen and Cullen Jour. Exp. Med., 1920, 32:547, 571.

distribution. If our knowledge was more extensive than it is, we should probably be able to describe a basal flora, characteristic of mankind under all conditions; a supplementary flora, varying in frequency, but ranging widely as normal parasites of man; and various species showing a restricted range or a temporary prevalence, conditioned by localized or transient environmental factors. This aspect of bacterial ecology has, however, received but little attention, except with regard to the incidence of a few important pathogenic species. If we include, among the normal flora of a particular region of the body, all those bacterial species which may be isolated from any considerable sample of apparently healthy persons, we must note that this flora varies widely, from time to time, and from place to place.

The nature of the intestinal flora, for example, depends on a number of factors, such as diet, gastric acidity, degree of peristalsis, external temperature, and so on. The predominant flora on a herbivorous differs from that on a carnivorous diet; in the upper part of the small intestine it is more copious in persons with gastric hypoacidity than in normal persons. Again, the flora of the nasopharynx is constantly changing both in the same individual and in the same community. At one time, pneumococci and Pfeiffer's bacilli may be present in large numbers in apparently healthy persons, whereas at another time they may be far less frequent. It must be realized, therefore, that, in the account which follows, we can make no attempt to give a complete description of the organisms that may be found in different parts of the body, or to indicate more than roughly their frequency and relative proportions.

The species found in the regions of the body which are normally colonized by bacteria include those generally accepted as pathogens, those generally accepted as saprophytes, and many that are intermediate. We know very little of the nature of the association between the bacterium and the healthy host tissue in any of these three groups. We may regard all typical members of a pathogenic species as virulent, and postulate a high level of immunity, perhaps specific, in order to explain the prevalence of the organism in the normal flora; or we may postulate a wide variation of virulence in the species, and assume a lesser virulence for the strains found in healthy tissue. For example, as we saw in Chapter 74, the "carrier" types of pneumococci appear to invade the lung tissue of carriers only when the host is subjected to debilitating influences, and the "infective" types may be carried by healthy people who have acquired an immunity to them (see, for example, Finland, Brown and Barnes 1940); and there are indications (see p. 1375) that the normal fusiform bacilli of the mouth may initiate ulcerative lesions in the mouth of vitamin-deficient animals. But except where there is an abnormally high specific antibody response to a species that occurs in the flora of normal tissues, any explanation in terms of a balance between the virulence of the "pathogen" and the resistance of the host is little more than a re-description of the observed state of affairs, though it serves to emphasize that the coexistence of bacterial with host cells is the result of an equilibrium between two sets of factors, and not merely a mutual indifference to each other's presence. And by analogy with a large number of instances of biological associations variously described as parasitism, saprophytism, commensalism or symbiosis, we may expect that the associations of saprophytes and healthy tissue cells are equally dynamic.

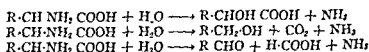
Moreover, equilibria exist not only between host and "normal" flora, but between the different species within the flora; and the association of various

and be broken down by enzymes concentrated there, the bacteria starve to death. Added food material such as peptone may be regarded simply as nutrient which makes possible the initiation of growth rather than as substances essential to the formation of proteolytic enzymes.

The evolution of ammonia which accompanies the bacterial decomposition of protein material is considerably curtailed if a fermentable carbohydrate is included in the protein-containing medium in which the organisms are grown. This phenomenon has been interpreted to indicate that carbohydrate exerts a "protein-sparing" effect on bacterial proteolysis analogous to its protein-sparing effect on mammalian metabolism. Precise work, however, has shown that the analogy is spurious. A diminution in ammonia found might be due to either a decreased production or an increased utilization, since ammonia is the chief source of nitrogen for the great majority of bacteria. The presence of readily available energy in the form of a fermentable sugar stimulates growth with a coincident increase in ammonia assimilated and, in consequence, the ammonia present is diminished.

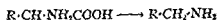
The Decomposition of Amino Acids. As pointed out above, with very few exceptions, such as the autotrophic organisms, all bacteria are able to utilize amino acids, and in most cases the decomposition of amino acids present in an actively growing bacterial culture proceeds rapidly. The enzymes concerned in the primary utilization of amino acids have been studied at some length, knowledge of them is reviewed by Gale.⁶⁷ Possibly these compounds may be used directly in some cases as building stones for bacterial protein; but in most instances some type of decomposition occurs which may liberate energy to the organism, and some of its products, such as ammonia, are the starting points of bacterial synthesis. Amino acids may be broken down in a variety of ways which may be summarized as follows:

(1) *Hydrolytic decomposition* which may result in the formation of a lower fatty acid and ammonia, of an alcohol, carbon dioxide and ammonia, or of an aldehyde, a lower fatty acid and ammonia by the following reactions



which are often brought about by aerobic organisms. The decomposition to an alcohol is frequently brought about by bacteria.

(2) *Decarboxylation with the formation of the corresponding amine* (the so-called ptomaines including pentamethyldiamine or cadaverine from lysine and tetramethylenediamine or putrescine from ornithine or arginine) is brought about by a variety of bacteria.



The bacterial amino acid decarboxylases have been studied in detail by Gale.⁶⁷ He has shown that six amino acids are commonly decarboxylated, lysine, ornithine, arginine, tyrosine, histidine and glutamic acid, all having in common a free carboxyl group in the one position, a free α -amino group, a free terminal polar group, and the natural *levo* configuration. It has also

⁶⁷ Gale Bact. Rev., 1940, 4:135, Advances in Enzymology, 1946, 6:1.

than in the healthy mouth; (5) "*Veillonella*"—said to be a normal inhabitant of the saliva (Langford *et al.* 1950); (6) Gram-negative bacilli, including members of the coliform and proteus groups; (7) Spirochaetes; these are almost invariably present between the gums and the teeth; several types have been described, such as *Treponema buccalis*, *Treponema dentium*, *Treponema intermedium* (Dobell 1912), *Treponema microdentium*, *Treponema macrodentium* (Noguchi 1912), Vincent's spirillum, and Miller's spirillum. Vincent's spirillum is often present in large numbers in the healthy mouth, usually accompanied by fusiform bacilli (see Brooke 1938); (8) *Actinomyces* (see Brooke 1938, Rosebury *et al.* 1944, and Chapter 14); (9) *Fusiformis* (see Brooke 1938 and Chapter 18); (10) Pleuropneumonia-like organisms (see Smith and Morton 1951); (11) Yeasts—mainly *Candida albicans* (Todd 1937, Lilienthal and Goldsworthy 1950). The flora of the mouth consists mainly of penicillin-sensitive organisms. By the use of penicillin pastilles most of these organisms can be eliminated. They are replaced by coliform bacilli and by yeasts (see Long 1947).

The intestinal flora of the breast-fed infant is fairly simple, consisting largely of *Lactobacillus bifidus*; this bacillus in the early weeks of life may constitute 99 per cent. of the total organisms in the faeces (Cruickshank 1925). There are in addition a few enterococci and Gram-negative coliform bacilli (Tissier and Dreyfus 1925). Snyder (1940), who followed the changes in faecal flora of infants from birth until some weeks after weaning, confirmed the predominance of *L. bifidus* in breast-fed infants, but found it also in 25 per cent. of the weaned infants. Coliforms and enterococci were also present in the breast-fed, but their frequency increased with weaning, and at the same time clostridia and members of the *Fusiformis* group became characteristic features of the faecal flora. Staphylococci of both the *albus* and the *aureus* species are often present, though usually in only small numbers (see Crowley *et al.* 1941, McFarlan *et al.* 1949, Martyn 1949, Buttiaux and Pierret 1949). They are probably derived from the infant's nose and from the mother's milk.

The intestinal flora of bottle-fed infants is not so simple. *L. bifidus* is uncommon; on the other hand, another member of the aciduric group of bacteria—*Lactobacillus acidophilus*—is usually present in large numbers (Tissier 1900). Different types of coliform bacilli, enterococci, Gram-positive aerobic spore-bearing bacilli and anaerobic bacilli, occur more or less abundantly. Two moderately pathogenic clostridia, *Cl. caputovale* and *Cl. difficile*, were first discovered in the stools of normal infants (Snyder and Hall 1935, Hall and O'Toole 1935, Snyder 1937).

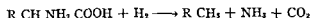
In the adult, numerous workers have found that the empty stomach is generally sterile. Immediately after a meal it contains numerous organisms, which have been ingested with the food; but these, with the exception of acid-resistant vegetative bacilli and sporing bacteria, appear to be killed off rapidly. If, however, the motility of the stomach is excessive, or the acidity is below normal, this sterilizing effect of the gastric juice is probably incomplete. Thus in cases of gastric disease—particularly of carcinoma—sarcinae, saprophytic bacilli, and other organisms may actually multiply in the stomach (Goodsir 1842, Oppler 1893). In the healthy adult the jejunum and upper ileum are practically sterile. How far this is attributable to the continuing effect of the gastric juice and how far, as Cregan and Hayward (1953) suggest, to some other antibacterial mechanism, it is at present impossible to say. In the lower ileum bacteria are often to be found.

been reported that aspartic acid is decarboxylated to β -alanine in symbiotic nitrogen fixation, and the decarboxylation of tryptophane has been reported also. It is of interest that pyridoxal phosphate is a coenzyme for the decarboxylases studied by Gale.

(3) Reductive deamination with the formation of saturated fatty acids and ammonia

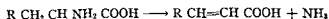


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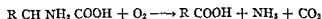
with the formation of the saturated hydrocarbon. These reactions are brought about by anaerobic bacteria which may, for example, decompose glycine to acetic acid and ammonia, or methane, carbon dioxide and ammonia.

(4) Deamination and desaturation at the α - β linkage with the formation of the unsaturated fatty acid and ammonia

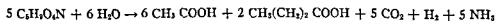


is also carried out by anaerobic bacteria.

(5) Oxidative deamination with the formation of ammonia, carbon dioxide and a fatty acid of one less carbon is brought about by aerobic organisms.

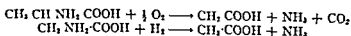


(6) Anaerobic oxidative deamination accompanied by the evolution of hydrogen and the disruption of the amino acid molecule has been described. The products of decomposition of glutamic acid have been determined



reaction probably proceeds by stages which are yet unknown. This type of decomposition is sometimes known as the "fermentation" of amino acids.

(7) The mutual oxidation and reduction of pairs of amino acids resulting in deamination and decomposition, undoubtedly an important source of energy to some obligate anaerobes and possibly facultative and aerobic organisms as well. Glycine, for example, is reduced to acetic acid and ammonia, and alanine oxidized to acetic acid, carbon dioxide and ammonia



The reactions are, of course, an oxidative and a reductive deamination respectively, but the significance lies in the fact that one amino acid may act as hydrogen acceptor in the oxidation of another. The paired oxidation-reduction of amino acids has been noted earlier in connection with respiration.

The aromatic amino acids are usually attacked first at the side chain, while the ring may or may not be broken. A number of organisms decarboxylate histidine to the physiologically active substance histamine and carry the decomposition no further. The breakdown of tryptophane to indol by some bacteria and not by others is taken advantage of in the biochemical differ-

found that *Chromo. prodigiosum* introduced by fistulæ into different parts of the intestine disappeared just as rapidly as when given by the mouth and made to pass through the stomach. Estimations of pH at different levels showed that the stomach contents had a pH approximately of 4-5, the duodenal of 6-7, the jejunal of 6-7.5, the ileal of 6-7, the cæcal of 5-7, and the descending colon of 5.5-7. In experiments *in vitro* a pH of about 4 was required to bring about a rapid reduction in the number of these organisms. This pH was met with only in the stomach. If, therefore, bacterial destruction occurs as rapidly in the intestine of the monkey as in the stomach, it is clear that acidity cannot be solely responsible. Bergeim described two antibacterial mechanisms in the cæcal contents of rats and man, one dependent on volatile fatty acids—formic, acetic and butyric—the other on H_2S . At the pH of the cæcal contents, butyrate from a high butter fat diet was found to act slightly on *L. acidophilus* or yeasts, and more strongly on *Bact. coli*, *Proteus vulgaris*, and salmonellæ. If rats on this diet were given $CaCO_3$ to raise the pH of the intestinal contents, the proportion of *Bact. coli* in the faeces increased. In concentrations that occurred naturally in the cæcum of rats and man, H_2S killed yeasts, and inhibited *Bact. coli* to some extent, but hardly affected *L. acidophilus* or the enterococcus (Bergeim 1940, Bergeim *et al.* 1941).

Provisionally we may conclude that in healthy persons the majority of the organisms ingested in the food and swallowed with the saliva are destroyed in the stomach by the gastric juice. The few surviving bacteria that pass through unharmed find the conditions in the duodenum and upper jejunum too acid to allow them to multiply. With their further passage down the intestine, they encounter progressively more alkaline conditions, and are able to proliferate accordingly (see Chapter 45).

The variations of intestinal flora within mammalian species may be very great. For example, the normal flora of the guinea-pig intestine was found by Crecelius and Rettger (1943) to be fairly simple; a lactobacillus resembling *L. acidophilus* constituted approximately 80 per cent. of the total cultivable flora, the remainder being accounted for chiefly by soil and air bacteria, yeasts, and an anaerobic sarcina. Coliform organisms and enterococci were seldom present. Again, according to Porter and Rettger (1940) the flora of the white rat on a normal mixed diet consists mainly of lactobacilli and coliform organisms; very few cocci are found. Lactobacilli were common in the stomach, where little else but yeasts were demonstrable. The bacterial content of the duodenum and jejunum was low, and no lactobacilli were present. Coliforms and lactobacilli were present in the lower parts of the small intestine, and abundant in the large intestine, where spore-bearing anaerobes also appeared. The lactobacilli were generally reduced by meat, to some extent by other types of high protein diet, and by several days' starvation. Except in the lower gut, it may be noted, there was little correlation between the pH of the gut contents and the predominating flora.

Origin of the Alimentary Flora.—Witkowski (1935), investigating the origin of the mouth flora, found that organisms could be demonstrated in the mouths of 49 out of 50 infants shortly after birth. These comprised mainly *Staph. albus*, coliform bacilli, Döderlein's bacillus, and streptococci. Examination of vaginal swabs from the mothers showed that these organisms were all present in the vagina in proportions corresponding fairly closely to those in the infant's mouth. It seems probable, therefore, that the initial flora of the infant's mouth is derived from the vagina of the mother. By 2-5 days after birth other organisms had appeared consisting, in decreasing order of frequency, of pneumococci, hæmolytic staphylococci, various types of streptococci, Friedländer bacilli, corynebacteria, sarcinae, and Gram-negative cocci. Comparison of these findings with the mouth flora

entiation of many species from one another. Tyrosine may be decomposed to phenol or broken down completely, and a number of organisms break the pyrrolidine ring of proline without difficulty. Owing to differences in ring structure, the decomposition of the aromatic amino acids becomes a series of special cases which cannot be considered further here.

The types of amino acid breakdown outlined above represent a generalization. No single amino acid has as yet been shown to undergo all of the decompositions nor is any single bacterial species capable of bringing about all of these transformations. On the other hand, the bacterial decomposition of an amino acid often involves not a single type of breakdown alone but a series of reactions consisting of several of these types. The nature of the products formed is dependent, to some degree, upon oxygen tension; some compounds such as hydroxy acids may be formed under aerobic conditions but are unstable under anaerobic conditions. The pH of the medium is also of considerable importance; for example, deamination occurs at an alkaline reaction and decarboxylation at an acid reaction. The fact that ammonia is almost always liberated is of considerable significance, for ammonium salts are utilized as a source of nitrogen by the great majority of bacteria.

Aerobic and anaerobic protein decompositions are often differentiated by the terms decay and putrefaction. Superficially there would appear to be some justification for regarding the two as separate processes—the justification being largely a matter of the unpleasant odors associated with the reduced products of anaerobic decomposition as contrasted with the lack of odor of the oxidized end products of the aerobic decomposition. Such a distinction has no reality, however, for the processes of decomposition are essentially the same in both cases. Both involve preliminary hydrolysis to amino acids and have further breakdown mechanisms in common. Oxidative deamination, for example, is commonly associated with aerobic organisms, but it also occurs in the oxidation-reduction of pairs of amino acids, a reaction brought about by many of the obligate anaerobes.

Synthesis of Nitrogen Compounds. Not only the autotrophic bacteria but also the most fastidious of the heterotrophes utilize ammonia and ammonium salts as sources of nitrogen. Although, as will appear, amino acids are assimilated directly, they also serve as a source of ammonia through preliminary deamination. The ammonia is taken up and used in the synthesis of amino acids, which are in turn condensed to form proteins.

Until relatively recently little has been known of the methods of synthesis of amino acids. The significance of the anabolic phase of carbohydrate metabolism has been discussed earlier and, as pointed out there, it seems probable that the formation of carbon chains and organic acids, of which keto acids are possibly the most important, constitutes an early stage in amino acid synthesis. Furthermore, the products of glycolysis, particularly pyruvic acid, may also be sources of starting material in amino acid formation. The next stage in synthesis is, of course, the addition of amino groups.

Of the enzyme-catalyzed deaminations, only two, catalyzed by aspartase and glutamic acid dehydrogenase, have been found to be reversible. It would appear, however, that a variety of amino acids could be synthesized from the corresponding keto acids by transamination, the amino group being donated

It is easy to pass from the conclusion that bacteria are unnecessary for the human organism to the conclusion that they are actively harmful. This is a transition that has been unjustifiably made. We have no space to discuss the question of *intestinal toxæmia*. The conception that numerous cases of ill-health and of actual well-defined disease processes are due to the absorption, from the intestine, of toxic products elaborated by proteolytic micro-organisms is a plausible one, but one that rests largely on unproven assumptions. It is the almost complete lack of experimental evidence that renders the discussion of this subject so fruitless at the present time (see Discussion 1913, Dudgeon 1926).

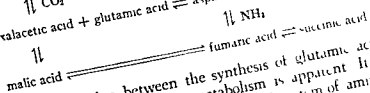
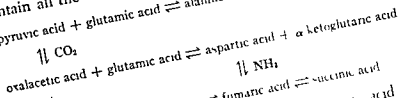
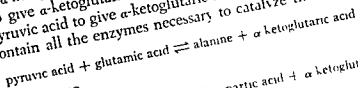
The earliest method attempted, in efforts to prevent the possible absorption of toxins from the intestine, was the replacement of the putrefactive flora—anaerobic spore-bearers such as *Cl. sporogenes*, *Cl. putrificum*, and *Cl. histolyticum*; aerobic spore-bearers such as *B. mesentericus*; and organisms such as *Proteus vulgaris* and *Ps. pyocyanea*—by an organism of the aciduric group known as *Lactobacillus bulgaricus*. These attempts failed completely. It has since been found impossible to implant this bacillus in the normal intestine; the conditions for its growth are unfavourable. Experimental work has shown that, in order to encourage the growth of a given type of organism in the intestine, it does not suffice to administer such an organism by the mouth, but that it is also essential to give by the mouth a sufficient quantity of a selective food material to enable it to flourish at the expense of other organisms. The replacement of a proteolytic by a saccharolytic flora—aciduric bacilli, *Cl. welchii*, and the enterococcus—can be largely accomplished by feeding on a high carbohydrate diet. In rats it has been found that by giving large quantities of lactose or dextrin in the diet, the intestinal flora can be so changed that it contains 90 per cent. or more of aciduric bacilli (Rettger and Cheplin 1921, Cannon and McNease 1923, Cruickshank 1928). These two carbohydrates are absorbed very slowly, and consequently pass into the large intestine. They are there acted upon by the aciduric bacilli—notably *L. acidophilus*—with the formation of a large amount of lactic acid; the presence of this acid is unfavourable to the persistence of the proteolytic bacteria. Without the supply of lactose, dextrin or milk the aciduric bacilli cannot be successfully implanted in the intestine, even though pure cultures are given in large quantity daily. In infants 12 per cent. of lactose must be added to whole cows' milk or to lactic acid milk in order to convert the mixed intestinal flora into a lactobacillary flora similar to that found in the stools of breast-fed babies (Gerstley, Howell, and Nagel 1932).

In old people, particularly those with hypochlorhydria, the small intestine is no longer sterile, the sugar in the diet is fermented before it reaches the large gut, and the lactobacilli are unable to thrive. In consequence the *fæces* contain salivary streptococci that have not been killed off in the stomach and larger numbers of putrefactive organisms than normally, but very few lactobacilli. Any benefit to be derived by such persons from drinking Yoghurt and other types of sour milk is not, as Metchnikoff supposed, due to the ingestion of the lactobacilli themselves but to the provision of milk sugar; this is not readily broken down till it reaches the large intestine, where the acid produced inhibits the development of putrefactive bacteria (Orla-Jensen *et al.* 1945). Whether sour milk has any advantage over sweet milk for this purpose is perhaps doubtful.

This is not the place to discuss the value of changing the intestinal flora in the treatment of constipation and other disorders, but we shall refer our readers

Synthesis of Nitrogen Compounds

by a dicarboxylic amino acid. Lichstein *et al*⁶⁸ have found that a number of bacteria can carry out this reaction between glutamic acid and oxalacetic acid to give α -ketoglutaric acid and aspartic acid, between glutamic acid and pyruvic acid to give α -ketoglutaric acid and aspartic acid, and between glutamic acid and pyruvic acid to give α -ketoglutaric acid and alanine. Bacteria such as *Bact. coli* contain all the enzymes necessary to catalyze the following reactions



The intimate relation between the synthesis of glutamic acid, aspartic acid and alanine with carbohydrate metabolism is apparent. It is possible that transamination systems are a part of a general system of amino acid synthesis but it is also possible that these reactions are a part of the respiratory mechanism concerned with the metabolism of four and five-carbon acids.

There is also fragmentary evidence of amino acid synthesis from studies on bacteria which require amino acids. There has, for instance, been some study of precursors, i.e., related compounds which can be substituted for the required amino acid, and it has been found, for example, that indol can be substituted for tryptophane in the growth requirements of typhoid bacilli requiring the amino acid, suggesting that such strains are unable to synthesize the indol ring. More detailed studies with *Neurospora* mutants have shown that anthranilic acid is a precursor of indol, and that indol is condensed with serine to form tryptophane.⁶⁹ Other studies have shown that the synthesis of arginine by *Neurospora* proceeds through ornithine and citrulline.⁷⁰ Still another approach is that of inhibition of amino acid formation by other amino acids or by analogues. The toxic effect of certain amino acid mixtures for the anthrax bacillus reported by Gladstone⁷¹ has been interpreted as an inhibition of synthesis, and similar observations have been made by other workers. The inhibition of methionine and methoxinine has been studied and it has been found that ethionine formation by analogues is inhibitory, and that the inhibitory activity is antagonized by methionine. It will be clear, however, that as yet there is only fragmentary information regarding the mechanisms of amino acid synthesis. The only general principles indicated, and these are no more than suggested, are those of reversible deamination and transamination.

As yet there is little information regarding the formation of peptide chains and proteins. There is reason to believe, however, that the tendency of glutamic acid to condense into peptides, and the tendency of β -aminobenzoic acid, linked

⁶⁸ Lichstein and Cohen *Jour. Biol. Chem.*, 1943, 151 349
 Umbreit, *ibid.*, 1945, 161 311.

⁶⁹ Tatum and Bonner *Jour. Biol. Chem.*, 1944, 154 129.

30 30

⁷⁰ Srb and Horowitz *Jour. Biol. Chem.*, 1944, 154 129.

⁷¹ Gladstone: *Brit. Jour. Exp. Path.*, 1939, 20 189

⁷² Lichstein, Gonsalus and Umbreit: *Proc. Nat. Acad. Sci.*, 1944, 157 85.

acid, of cocarboxylase from thiamin and of riboflavin from flavoproteins, takes place in the stomach. (For short reviews of this subject see Kon 1945, Elvehjem 1948, Johansson and Sarles 1949.)

As we have seen in Chapter 54, the fact that a substantial amount of the vitamin or food requirements of an animal may be satisfied by the bacteria in its alimentary canal affects the interpretation of a good deal of recorded work on experimental variations of diet. It also throws light on the association of deficiency states with diarrhoeal syndromes, and with prolonged therapy of intestinal infections with drugs like sulphaguanidine and succinyl sulphathiazole.

Intestinal bacteria may also destroy essential foodstuffs. Thus, in the absence of a more readily fermented carbohydrate, *Bact. coli* and a number of other intestinal species will decompose Vitamin C in media containing organic nitrogen. It is not known to what extent dietary Vitamin C is destroyed in the alimentary canal, but the hypothesis of its decomposition by bacteria is in accord with the observation that certain scorbutic patients respond to injected, but not to oral, Vitamin C (Young 1942, Young and James 1942, Young and Rettger 1943). Again, nicotinic acid may be utilized as the sole source of nitrogen by organisms of the *Pseudomonas* group and by certain chromobacteria, though in the absence of nicotinic acid, these organisms synthesize the vitamin (Koser and Baird 1944; see also Benesch 1945). The production of a vitamin by the intestinal flora will clearly be determined by a multiplicity of factors, of which the presence of vitamin-producing and vitamin-destroying bacteria is only one; and the determination of these factors presents an exceedingly complex problem in bacterial ecology.

A peculiarly fascinating aspect of the relationship of bacteria to nutrition was revealed by Stokstad and his colleagues in New York (1949) when they observed that the addition of aureomycin to the diet stimulated the growth of chicks. Similar observations were made by numerous other workers on pigs, chicks, and turkey poults, using aureomycin, terramycin, or penicillin (Wahlstrom *et al.* 1950, Coates *et al.* 1951, Sieburth *et al.* 1951, Larson and Carpenter 1952). The mechanism by which this effect is produced is still in doubt. It is thought by some workers that intestinal organisms detrimental to the host are suppressed by the antibiotic, and by others that by alteration in the intestinal flora organisms producing Vitamin B₁₂ or some other factor favourable to growth are stimulated.

The Normal Flora of the Respiratory Tract.

For our present purpose we may divide the respiratory tract into three sections: an upper part, including the anterior and posterior nares and the nasopharynx; an intermediate zone, common to the respiratory and alimentary tracts, including the oropharynx and the tonsils; and a lower part, including the larynx, trachea, bronchi and lungs.

The bacterial flora of the nasal passages differs in several respects from that of the nasopharynx. It is less copious: if swabs are taken from the nose and nasopharynx of a sample of normal persons, and plated on some suitable medium, the nasal swabs will give the lower colony counts, and the differences observed are often striking. Qualitatively, diphtheroid bacilli and staphylococci—both *Staph. aureus* and *albus*—are far more frequent in the nose than in the nasopharynx, whereas *Str. viridans*, indifferent streptococci, and Gram-negative cocci of the *N. pharyngis* type, are far less frequent. These non-hæmolytic streptococci and Gram-negative cocci appear to constitute the basal normal flora of the naso-

through a pterine nucleus to glutamic acid to give the vitamin pteroylglutamic acid (folic acid), are in some way intimately related to peptide and protein synthesis. This assumes considerable significance in view of the antagonism of *p*-aminobenzoic acid for the sulfonamides, and it is possible that these drugs may act through inhibition of synthetic reactions.

The Decomposition of Fats. From the quantitative point of view fats are not an important food source for bacteria. Bacterial lipases are not uncommon, however, and the action of bacteria on fat-containing media has some use as a differential character. Following hydrolysis the glycerol is readily fermented by a wide variety of bacteria, but the ability to decompose the higher fatty acids does not seem to be common and a number of these, such as palmitic, stearic, oleic and other acids, cannot be utilized as a source of carbon by many of the well known bacteria. The oxidative decomposition of fatty acids assumes some importance with regard to the development of rancidity of fat-containing foods.

NITROGEN FIXATION⁷²

The ability to use molecular nitrogen as a nutrient is, like the oxidation of ammonia and nitrite, a property of a limited number of bacterial species. Evidence of the biological basis of nitrogen fixation was reported in 1862, and it was later shown that the increase in organic nitrogen content of uncultivated soils could be prevented by sterilization or storing at a low temperature. Following the work of Winogradsky, Beijerinck, Hellriegel and Wilfarth and others it became apparent that atmospheric nitrogen is fixed by

- (1) free-living bacteria both
 - (a) anaerobic and
 - (b) aerobic and by
- (2) bacteria living symbiotically with leguminous plants.

The anaerobic organism, *Clostridium pastorianum*, isolated by Winogradsky in 1893, was the first nitrogen-fixing bacterium to be studied in pure culture. When cultivated under anaerobic conditions in a medium containing glucose but no nitrogen, the organism grows well, obtaining its energy through the fermentation of the sugar and its nitrogen from nitrogen gas. The amount of nitrogen fixed is proportional to the amount of glucose fermented—2.4–2.9 milligrams of nitrogen fixed per gram of glucose fermented in Winogradsky's experiments. The fixation of nitrogen is inhibited by the presence of ammonium salts in the medium and may be lost through continued cultivation on nitrogen-containing media. *Cl. pastorianum* is a spore-forming rod closely related to the bacteria of the so-called amylobacter group (so-called because many of them stain blue with iodine), organisms which ferment carbohydrate to butyric acid. The chief products of the fermentation of glucose by *Cl. pastorianum* are acetic and butyric acids and carbon dioxide and hydrogen. It has been found that the ability to fix nitrogen may be "restored" to some amylobacter species by a method of "soil passage" which restores this property to strains of *Cl. pastorianum* which have lost it through prolonged cultivation.

⁷² Biochemical nitrogen fixation is reviewed by Burk and Burris, *Ann. Rev. Biochem.*, 1941, 10:587; Burris and Wilson, *Ann. Rev. Biochem.*, 1945, 14:685; Wilson and Burris: *Bact. Rev.*, 1947, 11:41.

pathogenic species to vegetate in the nose than in the nasopharynx. Mouth-washing, in this series of cases at least, appears to be a less effective method of isolating the pneumococcus and the influenza bacillus, but equally effective for the hæmolytic streptococci and the Gram-negative cocci (see also Gundel and Linden 1931, Gundel and Okura 1933, Gundel 1933).

It must not be supposed that these are constant figures. They fluctuate widely both from time to time and from place to place. Thus the nasopharyngeal carrier rate of hæmolytic streptococci may vary from zero to 20 per cent. in a sample of the normal adult population subjected to repeated swabbing. The fluctuations in pneumococci and influenza bacilli are usually smaller (see also Burky and Smillie 1929).

Table 181 summarizes the results of a large survey by Straker, Hill and Lovell (1939), which included a group of adults sampled at intervals over a period of seven years. In children, the pneumococcal carrier rates tended to be higher than those recorded in the table, but otherwise the rates were not significantly different.

TABLE 181

SHOWING THE CARRIER RATES FOR CERTAIN BACTERIAL SPECIES IN THE NASOPHARYNX AND NOSE FOR A GROUP OF ADULTS IN AN URBAN POPULATION

Organism.	Nasopharyngeal Carrier Rate	Nasal Carrier Rate.
	Per cent.	Per cent.
<i>Str. pneumoniae</i>	20-40	5-15
Hæmolytic streptococci	5-15	< 1
<i>H. influenzae</i>	40-80	5-10
<i>N. meningitidis</i>	5-20	0-4
Gram-negative cocci other than <i>N. meningitidis</i>	90-100	0-15

The rates varied to some extent with the season. In the nasopharynx, pneumococci and to a slight extent *H. influenzae* were found more frequently in cold, damp seasons than in the dry hot periods; in the nose a similar, but more definite seasonal variation occurred. There was some evidence of an association between these two organisms, whose colonization of the nasopharynx appears to be fairly constant; in the damper colder seasons, they appeared more frequently in the nose of living persons, and in the trachea of persons who had died from a variety of causes. That is, in the summer months the organism was confined largely to the nasopharynx, but in late winter and early spring it tended to colonize downwards into the trachea and forward into the nasal cavity.

The carrier rates for hæmolytic streptococci in the throat are substantially the same in other countries as in Great Britain (see, for example, Bryce and Tewsley 1938, Wu 1941, Boisvert 1942, and Chapter 66). An association of high carrier rates with the possession of tonsils, in both children and adults, has been noted by a number of workers. Thus, Burpee (1937) in an institutional survey records a rate of 27.3 per cent. in persons with tonsils, and 10.3 per cent. in those whose tonsils had been removed (see also Chesterman and Scandrett 1940, Pike and Fashena 1946). Of the hæmolytic streptococci isolated from the throat, a large proportion belong to Lancefield's Group A. The proportion varies from 20-70 per cent (see, for example, Fleming 1939, Pomales-Lebrón 1940, Williams and Harper 1944), some of the remainder belonging to Lancefield's other groups. It is noteworthy that Group A streptococci colonize the tonsils more readily than other hæmolytic

Nitrogen Fixation

on laboratory media. It is probable that all these organisms constitute a relatively homogeneous group whose natural habitat is soil

The aerobic nitrogen-fixing bacteria were discovered by Beijerinck in 1901 and named by him *Azotobacter*. Two species were originally suggested: *Azotobacter chroococcum*, a pigmented (deep brown to black), nonmotile organism found predominantly in soil, and *Azotobacter agilis*, a motile form producing a soluble green pigment and found in water. Two other species have been found in this country, *Azotobacter vinelandii* and *Azotobacter beijerinckii*. Although one or two additional species have been described, these four have until recently, been the generally recognized members of the genus *Azotobacter* as typical of the group. This species has a world wide distribution; the other three are apparently more restricted although *A. agilis* may have a wider distribution than formerly thought. All fix nitrogen in alkaline

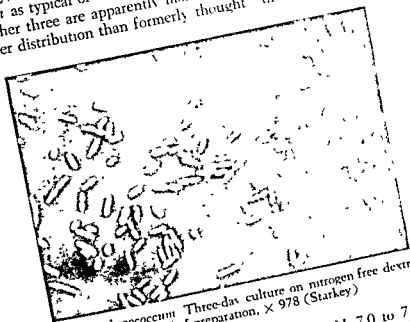


fig. 19. *Azotobacter chroococcum*. Three-day culture on nitrogen free dextrose medium Nigrosin relief preparation, $\times 978$ (Starkey)

environments, fixation proceeding most rapidly at pH 7.0 to 7.5. Starkey⁷¹ has reported the isolation of a species designated as *Az. indicum* from acid soil which fixes nitrogen when grown in an acid (pH as low as 3.0) medium. *Azotobacter* survives for long periods of time, as long as twenty years, and still is able actively to fix nitrogen.

In addition to reaction, a number of other factors govern nitrogen fixation by these organisms. Phosphate, calcium and oxidizable carbon compounds (mannitol or propionate are usually supplied in laboratory media), in addition to certain metals such as iron and molybdenum,⁷² are necessary for fixation, and the numbers of these organisms in a given soil may often be increased by the addition of chalk, lime, phosphate or carbohydrate, depending upon the deficiencies of the particular soil.

Like *Cl. pasteurianum*, *Azotobacter* does not fix nitrogen in the presence of adequate amounts of nitrogen compounds in the medium but, unlike the anaerobe, does not lose the ability to fix nitrogen through continued cultivation.

⁷¹ Starkey: Science, 1939, 89 267. See also Starkey and De Soil Sci., 1939, 47 329
⁷² Cf. Burk. Ergebnisse der Enzymforschung, 1934, 3 23

and 24 months (see also Ludlam 1953). Observations made by numerous workers suggest that in maternity units infants become infected with *Staph. aureus* more often from nurses and from the dust of the nursery than from their mothers.

Various workers have described the presence of small Gram-negative, filter-passing, anaerobic bacilli, in suspensions obtained by washing out the nares, nasopharynx and oropharynx with sterile broth (Olitsky and Gates 1921a, b, Olitsky and McCartney 1923, Mills, Shibley and Dochez 1928, Garrod 1928, Burky and Freese 1931). The evidence suggests that these strains may be differentiated into several distinct types; but they have not yet been submitted to detailed study from the systematic point of view. The so-called "*Bacterium*" *pneumosintes*, at one time regarded as a possible cause of epidemic influenza, apparently belongs to this group. The information at present available on the relative frequency of these bacilli in normal persons, and in those suffering from colds, does not indicate that they play any significant rôle in such infections. Garrod (1928) also described the isolation, by the same technique, of minute, Gram-negative, filter-passing, anaerobic cocci.

The Normal Flora of the Vagina.

An important distinction must be drawn between the flora of the vulva and vestibulum on the one hand and that of the vagina proper on the other. Except immediately after parturition and during the first few days of the puerperium, the vaginal flora is quite distinct from that of the vulval flora.

The *vulva* of the newly-born child is sterile; organisms make their appearance in about 7 to 8 hours. The normal flora of the vulva is a rich and varied one, and depends largely on the organisms present in its immediate environment. According to Wegelius (1909) it consists of: (1) Obligatory aerobes and aerophilic bacteria, of which the chief types are pseudo-diphtheria bacilli and *M. tetragenus*. (2) Coliform bacilli. (3) Facultative anaerobes, usually more or less susceptible to acid. (4) Bacilli derived from the vagina, including yeasts. (5) Obligatory anaerobes. On agar plates incubated aerobically the commonest organisms to form colonies are staphylococci, diphtheroids, enterococci, sarcinae, and coliform bacilli. Besides these organisms, yeasts—*Oidium* and *Saccharomyces*—are very common; and the smegma bacillus is not infrequently demonstrable in smear preparations stained with Ziehl-Neelsen. Under anaerobic conditions of cultivation numerous colonies appear, consisting of organisms that have as yet been only imperfectly studied. Pathogenic bacteria are uncommon.

The normal flora of the *vagina* seems to depend largely on the glycogen content of the vaginal epithelium which, in its turn, is dependent on ovarian activity (Miura 1928, Cruickshank and Sharman 1934). The vagina of the newly born child is sterile; organisms make their appearance in 12-24 hours. At first they consist of staphylococci, enterococci, and diphtheroids, but these are often replaced in 2 or 3 days by a practically pure culture of Döderlein's bacillus (see Chapter 31). At this time glycogen is demonstrable in the vaginal epithelium, and the vaginal secretion itself is acid. The occurrence of glycogen appears to be due to the presence of œstrin derived from the maternal circulation. Soon this is excreted in the urine glycogen is no longer demonstrable in the epithelium,

tion on nitrogen-containing media. Although *Azotobacter* fixes nitrogen in the free-living state, it is not infrequently found in nature living symbiotically with higher plants. *Chlorella*, for example, may grow in association with the bacterium, the former assimilating carbon dioxide and the latter molecular nitrogen.

The mechanism by which nitrogen is fixed is obscure. Winogradsky suggested that *Cl. pastorianum* might bring about a direct combination of nitrogen with nascent hydrogen liberated in the fermentation of glucose to form ammonia. It has been generally supposed that the fixation of nitrogen is an endothermic process, but Burk⁷⁵ has pointed out that fixation might take place by means of exothermic reactions either in the presence of hydrogen arising from the fermentation of glucose or formate, or as an oxidation in the presence of free oxygen.⁷⁶ Wilson and Burris⁷² have reviewed more recent evidence



Fig. 20. Colonies of *Rhizobium radicicola* on nutrient agar. Twenty-four hour culture, $\times 3$.

regarding the mechanism of fixation and conclude that ammonia is the most likely intermediate, arising from the reduction of nitrogen by substrate hydrogen. As in other phases of bacterial chemistry, the temptation to assume a free radical chemistry is strong, but there is as yet insufficient evidence to justify such an assumption.

The efficiency of the fixation process is variable depending upon the partial pressure of oxygen. The high respiratory rate of *Azotobacter*, $QO_2 = 2000$ to 4000, the highest recorded for any living cell, is at a maximum at 10 to 15 per cent oxygen, falling off sharply on either side of this optimum. Maximum nitrogen fixation, however, takes place at 4 to 5 per cent oxygen, and the efficiency of the fixation process increases with decreasing oxygen pressure. Under optimum conditions nitrogen is fixed with an efficiency of approximately 11 per cent of the maximum theoretically attainable, a figure which falls off to about 1 per cent under atmospheric conditions.

⁷⁵ Burk: Jour. Gen. Physiol., 1927, 10:559.

⁷⁶ An oxidation to HNO_3 . If the concentration of HNO_3 is less than 0.1 molar, ΔF has a positive value.

human beings and to rabbits, but potentially pathogenic *Staph. aureus* is not infrequently found (Koch 1908). In moister parts, like the axillæ and inguinal folds, Gram-negative bacilli often predominate.

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The Symbiotic Nitrogen-Fixing Bacteria

The Symbiotic Nitrogen-Fixing Bacteria. The enrichment of a soil by the cultivation on it of leguminous plants such as soy beans, vetch, peas, clover and the like has been known throughout recorded history. Although such plants were early (1838) shown to take up atmospheric nitrogen, the relation between nodule formation and nitrogen fixation was first conclusively demonstrated by Hellriegel and Wilfarth in 1888 and the bacteria present in the nodules were cultivated by Beijerinck in the same year. These organisms, called by him *Bacillus radicumicola* and now known under the generic name of *Rhizobium*, were shown through subsequent work to fix atmospheric nitrogen when living symbiotically in the root nodules. Although the bacteria occur free in the soil, nitrogen is not fixed except in intimate association with the host plant.

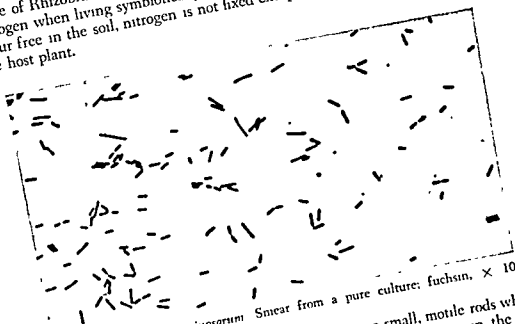


Fig. 21. *Rhizobium leguminosarum* Smear from a pure culture; fuchsin, $\times 1050$.

The bacteria in pure culture on artificial media are small, motile rods which do not form spores. They show a high degree of pleomorphism in the root nodules, however, small oval forms and branching forms (*bacteroides*) are found admixed with the rod forms. Some workers regard these bacteria as a single species, *Rhizobium leguminosarum*, which is made up of a variety of strains differing from one another immunologically and in their ability to infect the various species of leguminous plants. These strains fall into two groups, however, one having peritrichous flagella and being somewhat more active biologically than the other, which has monotrichous flagella. Bergey (1948) recognizes six species of *Rhizobium*, viz., *Rh. leguminosarum* (legumes), *Rh. phaseoli* (beans), *Rh. trifolii* (clover), *Rh. lupini* (lupine), *Rh. japonicum* (soy beans), *Rh. meliloti* (clover). *Rh. radicumicola* is regarded as synonymous with *Rh. leguminosarum*. As a group they appear to be closely related to *Bacterium aerogenes*, Friedlander's bacillus and similar organisms. An organism known as *Bacterium radiobacter* is often found associated with the nodule bacteria but does not fix nitrogen.²²

²² For a study of this organism see Hofer Jour. Bact. 1941, 41:193

(3) With avirulent bacteria, which have little power of multiplication within the tissues, this mechanism results in a rapid and permanent clearing of the circulation. With virulent bacteria an initial partial clearing is followed by a stage of bacterial multiplication in the foci to which the bacteria have been removed, resulting in a secondary invasion of the blood stream. The fate of the host is determined by the balance between this bacterial multiplication and the efficiency of the clearing mechanism. With slightly or moderately virulent strains there is a secondary bacteræmia followed by a secondary clearing. With highly virulent strains the secondary bacteræmia increases and the animal dies of an acute septicæmia. Even when secondary clearing occurs, and the animal remains in apparent health, foci of infection may remain in the various histiocyte depôts, and living bacteria may persist in these situations over long periods of time.

(4) When bacteria get into the body by other routes they are dealt with in part by local mechanisms of the same general character as that described under (3). The extent to which invasion of the blood stream occurs depends upon the anatomy and functional activity of the tissues first invaded, and upon the virulence of the invading organism. The blood stream is invaded very rapidly after intraperitoneal inoculation, less rapidly after intramuscular inoculation, and still less rapidly after subcutaneous inoculation. In all cases the passage of the invader from the primary lodgment to the blood stream is mainly by way of the lymphatics, and the histiocyte depôts in the regional lymphatic glands play a prominent part in the removal of the invading bacteria. When relatively avirulent bacteria gain access to the tissues at some site from which passage to the blood stream is slow, infection may never pass beyond the first line of defence in the regional lymphatic glands, but here, as in the case where a generalized infection has become established, localized foci of infection may persist over long periods of time.

(5) In addition to this process of removal and digestion by phagocytic cells, a direct bactericidal action of the serum plays a part in ridding the tissues of bacteria. The importance of this purely humoral immunity appears to differ widely in different bacterial infections; but it is probably always subsidiary to the phagocytic mechanism, and is seldom, if ever, the main factor in defence.

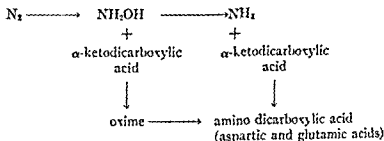
(6) The efficiency of the clearing mechanism of a normal animal may be increased, *vis-à-vis* a particular bacterial parasite, by active immunization with a killed culture of that particular organism. The immunity so produced is specific in the serological sense. The actively immunized animal deals with a virulent strain of the bacterium against which it has been immunized in the same way as a normal animal deals with an avirulent or slightly virulent strain of the same bacterial species.

(7) Immunity of this type may be passively transferred by the injection into a normal animal of the serum obtained from an animal that has been actively immunized.

(8) The antibodies by which this passive immunity is conferred are those that unite with the surface antigens of the virulent bacteria; and in order to induce an effective active antibacterial immunity the immunizing agent employed must contain these surface antigens.

(9) The efficacy of humoral immunity in any part of the tissues is determined in the first place by the existing concentration of antibody on the cells or in the tissue fluid, and the availability of circulating antibody; and secondly by the presence, in optimal amounts, of the reactants, both fluid and cellular, necessary

Rhizobium occurs only in association with the host plant, the mechanism of fixation is somewhat better understood than in the case of the free-living nitrogen fixers, chiefly through the work of Virtanen and his colleagues. It has been found that not inconsiderable quantities of nitrogen are excreted by the root nodules during the early stages of growth in the form of glutamic and aspartic acids and β -alanine together with a small quantity of the oxime of oxalacetic acid. It appears that nitrogen is fixed as hydroxylamine, which in turn condenses with oxalacetic acid supplied by the plant. The oxime is then reduced to aspartic acid, which serves as a starting material for the synthesis of other amino acids. The occurrence of glutamic acid has been taken to suggest that hydroxylamine is also reduced to ammonia, but is also compatible with the assumption that nitrogen is fixed as ammonia. The β -alanine found presumably arises through decarboxylation of the aspartic acid. The course of the fixation process may be expressed thus:⁷⁸



Plants other than the leguminosae show nodule formation which, in some cases, is associated with nitrogen fixation. A number of species of alder (*Alnus*), for instance, have root nodules and there is evidence that nitrogen fixation occurs. Nodule formation is not confined to roots: some plants, the Rubiaceae and Dioscorea, show bacteria-containing nodules on the leaves, and in one instance these have been shown to fix atmospheric nitrogen.

It is open to question whether bacteria other than those described above are able to fix nitrogen. Fixation has been reported for a variety of organisms such as the pneumococcus, but the evidence is inconclusive and fragmentary. It has been shown,⁷⁹ however, that the blue-green alga *Nostoc* is able to fix atmospheric nitrogen.

THE NUTRITIVE REQUIREMENTS OF BACTERIA⁸⁰

It is already apparent that bacteria as a group include a wide variety of physiological types, ranging from the photosynthetic and autotrophic forms to those which metabolize carbohydrates and organic nitrogenous substances by means of complex enzyme systems. The food requirements of these organisms are equally diverse, some being able to utilize inorganic compounds of carbon and nitrogen while others require organic compounds of varying de-

⁷⁸ Virtanen. *Ann. Rev. Microbiol.*, 1948, 2 485.

⁷⁹ *Ann. Rev. Microbiol.*, 1948, 2 485. *Bot. Gaz.*, 1937, 98:433.

been reviewed by Knight. Medical Research Council 1936 to 1938 by Koser and Saunders: *Bact. Rev.*, 1938, articles of bacterial metabolism by various authors in *Ann. Rev. Biochem.*, *Advances in Enzymology*, and *Ann. Rev. Microbiol.*, and most recently by Koser. *Ann. Rev. Microbiol.*, 1948, 2 121.

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Nutritive Requirements

degrees of complexity The kinds of food materials that must be supplied to an organism may be considered to fall into three categories.

- (1) compounds that can be oxidized by the organism and thereby serve as sources of energy,
- (2) compounds that serve as building stones or as precursors to building stones in the synthesis of bacterial protoplasm, and
- (3) substances which, for want of a better name, are designated as growth accessory substances or vitamin-like substances and which, in many cases, appear to function as components of catalytic systems in the economy of the organism

Although such a classification of food materials is convenient for purposes of exposition, sharp distinctions are sometimes difficult to make, as will appear.

In general, the utilization of a given substance as a food by an organism is dependent upon two factors. One, perhaps the more obvious, is the possession by the organism of enzyme systems which make possible the decomposition or assimilation of the substance in question. Cellulose, for example, is not a food for organisms which are unable to decompose it. The second factor is the concentration of the substance, which may vary from a lower limit below which the bacterium is unable to grow to an upper limit beyond which additional material is simply excess. Very high concentrations may actually be toxic, viz., the preservation of foods in syrups. Butterfield²¹ has shown that between these two limits a regular relation exists between the amount of bacterial growth and the concentration of nutrient material, if the logarithms of the maximum numbers of bacteria are plotted against the logarithms of the concentrations of food, the points fall on a straight line. The maximum numbers of bacteria are, in turn, dependent upon the size of the individual organisms, the relation between size and numbers of individuals being inverse—the smaller the bacterium the greater the number of individual cells. When the volume of cells is taken into consideration, however, it appears that a given volume of bacterial substance is formed in a given concentration of nutrient, the larger numbers of small organisms being but a consequence of wrapping the same amount of protoplasm in smaller packages.

Oxidizable Substances. The compounds that may serve as sources of energy to the growing bacterial culture have already been discussed in connection with the mechanisms of oxidation used by these organisms. Suffice it to say that the autotrophic forms require as food material the inorganic compounds of nitrogen, sulfur and iron that they can oxidize, and that such compounds may be regarded as nutriment in the same way that carbohydrates and amino acids are regarded as foodstuffs for the heterotrophic forms. The autotrophic bacteria, however, exhibit a high degree of specificity with respect to oxidizable substances which has no counterpart in the metabolism of the heterotrophic microorganisms. The latter, both as a group and as individual species, may obtain energy from a great variety of organic compounds of carbon and nitrogen. As long as such compounds are susceptible to attack by the enzyme systems of the bacterium, they may serve as food materials.

"Building Stones." The second group of food substances, those that serve as building stones, may be considered as sources of the chemical ele-

²¹ Butterfield Pub. Health Repts., 1929, 44:2865.

mesenteric glands or the liver; a few may perhaps make their way to the blood stream, from which they will be removed by phagocytic cells. In support of this possibility is the observation that the muscles and viscera of apparently healthy cattle and pigs not infrequently contain bacteria (Zwick and Weichel 1911, Reith 1926). Different animals, however, seem to vary in this respect. Schweinburg and Sylvester (1953), for example, found that the organs of rats, guinea-pigs, and golden hamsters were sterile, but that 80 per cent. of samples of tissues from dogs and 40 per cent. from rabbits yielded clostridia—mainly *Cl. welchii*.

The now common diagnostic practice of sternal puncture also offers a means of exploring the bacterial content of the reticulo-endothelial system in healthy persons. So far, its bacteriological use has been confined to culture of marrow samples as an aid to diagnosis in infections where the blood culture is sterile. A wide field of experimental work is open to the student who is willing to study the distribution of living organisms in the tissues of apparently normal animals.

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ments which, in appropriate and often highly complex combinations, make up the bacterial protoplasm. Chief among these elements are carbon and nitrogen which the organism may be able to assimilate in a variety of forms. The heterotrophic microorganisms require organic compounds as sources of carbon but do not appear to be particularly fastidious as to the nature of these compounds as long as they may be attacked by the bacterial enzymes. An organism may, for example, assimilate some of the carbon of a fermentable sugar or it may grow normally in the presence of one or more amino acids in mineral salt solutions. The apparent relative unimportance of the nature of the carbon compound suggests that bacteria may break down all manner of carbon compounds to one or more substances which they use as a starting material for the synthesis of cell substance. The intimate relation between glycolysis and the synthesis of carbohydrate and amino acids has been pointed out earlier. The source of nitrogen is either ammonium salts or ammonia liberated by deamination of amino acids supplied in the medium.

The Essential Elements. Elements other than carbon and nitrogen are a part of the bacterial protoplasm and must, of necessity, be supplied in a culture medium. Oxygen is, of course, contained in carbohydrates and amino acids as well as available in the gaseous form to all but the obligate anaerobes. Of the other elements, phosphorus predominates quantitatively because of the high nucleic acid content of the cells. It is generally supplied in the form of phosphate and probably may be assimilated by the organism in the form of other inorganic compounds. A number of other elements, such as iron, magnesium, calcium, etc., have been shown to be necessary to bacterial growth, and certain organisms may have special requirements as in the case of *Azotobacter*, which requires the presence of elements such as molybdenum and vanadium for nitrogen fixation (which in this case is growth, since the nitrogen is fixed as bacterial protoplasm). It has been found also that iron concentration is of paramount importance in toxin formation by the diphtheria bacillus, the optimum amount being 0.14 micrograms per milliliter.⁸² Similar results have been obtained in studies of the formation of tetanus toxin.⁸³ Iron concentration has also been shown to determine the type of sugar fermentation produced by *Cl. welchii*; with adequate amounts of iron, it is of the acetic acid-butyric acid type with not more than 20 per cent conversion to lactic acid, but with iron deficiency very little volatile acid is formed and 85 per cent of the sugar is converted to lactic acid.⁸⁴ Probably a great number of chemical elements go to make up the bacterial cell substance and are, therefore, necessary parts of the food supply, but in the great majority of cases they are required only in the minute traces with which many chemical compounds of the highest purity are contaminated. Obvious technical difficulties have prevented intensive study, but it is quite likely that all of these "biologically rare" elements may be assimilated in the form of inorganic compounds.

The Essential Amino Acids. Bacteria not only require elements such as carbon and nitrogen, but many require them in the form of preformed molec-

⁸² Pappenheimer and Johnson: Brit. Jour. Exp. Path., 1936, 17 335, Pappenheimer *ibid.*, 1936, 17 342.

⁸³ Mueller and Miller: Proc. Soc. Exp. Biol. Med., 1940, 43.389.

⁸⁴ Pappenheimer and Shaskan: Jour. Biol. Chem., 1944, 155.265.

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Bacterial Vitamins

ular structures. It has been pointed out that the failure of certain bacteria to grow in mineral salt solutions containing fermentable carbohydrate and ammonia might be accounted for on the assumption that such organisms lacked the ability to synthesize certain molecular structures, such as those contained in certain amino acids, and, in consequence, such structures might have to be supplied preformed. The basic assumption is, of course, analogous to the concept of "essential" amino acids in animal nutrition. A considerable quantity of evidence indicates that a number of bacterial species require the presence of certain amino acids in order to grow in mineral salt solutions. One strain of the diphtheria bacillus, for example, has been found to require a mixture of tryptophane, cystine, methionine, valine, histidine, glycine and glutamic acid, while another requires leucine, methionine, valine and glutamic acid. *Clostridium botulinum* requires cystine, leucine, lysine, glycine and proline, and another anaerobe, *Clostridium sporogenes*, requires tryptophane, cystine, leucine, methionine, valine, tyrosine, histidine, arginine and phenylalanine. The amino acid requirements of streptococci are similarly complex. The requirements of some other organisms are not so elaborate, some strains of the typhoid bacillus, for instance, will grow in a mineral salt solution plus tryptophane, while others need no amino acids. The significance of observations such as these is not altogether clear at the present time. It is assumed, of course, that such required amino acids are directly assimilated as building stones which the organism is incapable of synthesizing as indicated above; but the demonstration of the oxidation reduction of pairs of amino acids by obligate anaerobes indicates that these compounds may sometimes function as energy-yielding compounds rather than building stones. Furthermore, analytical evidence⁶⁶ suggests that the apparent necessity for the inclusion of amino acids in mineral salt solutions may not be indicative of a lack of synthetic abilities on the part of the microorganisms.

The direct assimilation of amino acids by bacteria has been shown by Gale and his associates,⁶⁷ who concluded that an associated fermentation of glucose is necessary for the passage of glutamic acid across the cell membrane, and for the passage of lysine out of the cell. The relation of such assimilation to the amino acid requirements of bacteria is not clear, but it may be noted that here again an intimate relation between glutamic acid and glycolysis is indicated. Indeed, further studies on glutamic acid assimilation have suggested that antibacterial substances such as sulfathiazole, triphenylmethane dyes and surface active compounds interfere either directly or indirectly with its assimilation.⁶⁸ an observation perhaps to be related on the one hand to the activity of glutamine as a bacterial vitamin (see below), and on the other to its possible role in the synthesis of amino acids by transamination reactions.

Growth Accessory Substances or Bacterial Vitamins. The third group of food materials, the growth accessory substances, consists of organic compounds which are necessary to growing bacteria only in minute amounts. Presumably these substances cannot be synthesized by the cell, thereby resem-

⁶⁶ Burrows Jour Inf Dis., 1933, 52 126.
⁶⁷ Burrows Jour Inf Dis., 1939, 61 145.

⁶⁸ Gale et al Jour Gen Microbiol., 1947, 1 53, 77, 86.
⁶⁹ Gale et al Jour Gen Microbiol., 1947, 1 299, 314, 327.

CHAPTER 91

THE BACTERIOLOGY OF AIR

IN Chapter 59 we have already discussed the apparently conflicting views of Cornet and of Flugge on the respective parts played by dust and by droplets in the spread of pulmonary tuberculosis. More recently observations in this country and in the United States have focused attention on the part played by aerial infection in the causation of other respiratory diseases. Work is now proceeding actively on aerial microbiology, and particularly on the ways in which spread of infection by dust and droplet nuclei can be controlled. We shall attempt to give a brief résumé of the present position, and refer our readers for a fuller account of the earlier observations to the Symposium on Aerobiology held at Chicago in 1942 (Report 1942), and for more recent reviews and studies to Rosebury's (1947) monograph on experimental air-borne infection, to the report of the Committee on Research and Standards (Report 1947), to the report of the Medical Research Council (Report 1948), and to papers by Williams (1948, 1949a, 1951).

Outdoor Air

The degree of bacterial contamination of the outside air depends on a number of factors, such as the density of the human and animal population, the amount of vegetation, the nature of the ground or soil, the temperature and humidity of the atmosphere, the extent and direction of wind currents, and so on. Aeroplane surveys (see Proctor 1934, 1935, Proctor and Parker 1938) show that bacteria in the upper air consist largely of aerobic spore-bearing bacilli, and to a much less extent of organisms belonging to the *Achromobacter*, *Sarcina*, and *Micrococcus* groups, suggesting that they are derived mainly from soil and surface dust. Their numbers are much the same over land and sea (Pady and Kelly 1953). These organisms can be carried vertically for several miles into the air, and it is possible that they may also travel long distances horizontally. It is conceivable therefore, that certain resistant pathogenic bacteria like the anthrax bacillus, and viruses like the virus of foot-and-mouth disease, might be transmitted from one area or country to another, and serve, on again reaching the ground, as infective agents for man or animals. There is, however, no evidence to prove that this is more than a possibility. On the contrary, most of our observations suggest that infective material is seldom carried for more than short distances by air, or that, if it is, it is too diluted to cause recognizable disease. Though autotrophic bacteria may possibly undergo some multiplication in cloud areas, where moisture and traces of gases such as NH_3 and CO_2 are present, it seems extremely improbable that growth of pathogenic organisms can occur in air. For the medical bacteriologist the importance of the flora of the outside air still remains to be

bling the essential amino acids but differing from them in that the growth accessory substances apparently function in the cell economy as catalysts rather than building stones for cell substance proper. The search for such substances goes back over many years, even antedating the discovery of the vitamins of mammalian physiology. The great majority of investigations were based upon a general type of experiment in which a solution which did not support the growth of the bacterium under consideration was supplemented by extracts of various plant and animal tissues. Since many of these extracts permitted growth in the otherwise inadequate solution, it was assumed that they contained active substances which were variously termed biocatalyzers, growth hormones and the like. The interpretation of experiments carried to this point but no further is exceedingly difficult. In very recent years, however, more precise information regarding these substances has become available.

It is of particular interest that the majority of these substances have proved to be identical with various components of the vitamin B complex, suggesting a close relation between the metabolic processes of organisms as diverse as bacteria on the one hand and mammals and other higher animals on the other. It is not inappropriate, therefore, to refer to these substances as *bacterial vitamins*. Other substances required by bacteria and included in this category are glutamine, purines such as adenine or uracil, pimelic acid, and others. The requirements of a number of bacteria have been worked out in some detail⁸⁹ but it should be noted that there may be, and often is, considerable variation from strain to strain of the same species.

The first bacterium to be studied in detail in this respect was the diphtheria bacillus which Mueller⁹⁰ found to require nicotinic acid and β -alanine, the latter presumably serving as a precursor of pantothenic acid; some strains require pimelic acid. *Staphylococcus aureus* was similarly studied by Knight⁹¹ and found to require nicotinic acid and thiamine and, for anaerobic growth, pyruvic acid and uracil had to be supplied. It has been found by others that certain strains require biotin. Similarly, the lactic acid bacteria have been found to require riboflavin, pantothenic acid, nicotinic acid, pyridoxine and other as yet unidentified substances, and the hemolytic streptococci must be supplied with pantothenic acid, pyridoxine, thiamine, nicotinic acid, glutamine and certain purines.

In other instances precise study has made possible an understanding of growth requirements already known. Thus, in the case of the hemophilic organism, the influenza bacillus, it has long been known that two substances present in fresh blood media are required. One of these, the "X factor," is heat-stable and associated with hemoglobin, while the other, the "V factor," is heat-labile and found in yeast and various vegetable extracts as well as in blood. It is now known that the X factor may be replaced by hematin and the V factor by coenzyme I or II.

Other bacteria, however, have much simpler nutritive requirements. The typhoid bacillus, for example, does not require added growth accessory sub-

⁸⁹ For detailed reviews see Peterson and Peterson Bact. Rev., 1945, 9, 49, Koser: Ann. Rev. Microbiol., 1948, 2, 121.

⁹⁰ Mueller. Bact. Rev., 1940, 4, 97.

⁹¹ Knight: Biochem. Jour., 1937, 31, 731, 966; *ibid.*, 1938, 32, 1241.

fallen to any appreciable extent. On evaporation, they become converted into what Wells calls "*droplet nuclei*," which behave very much like minute particles of smoke. In absolutely still air they fall at the rate of probably 1 to 3 feet per hour, but in the ordinary atmosphere they remain suspended almost indefinitely,

TABLE 182

EVAPORATION TIME OF WATER DROPLETS IN UNSATURATED STILL AIR AT 22° C
(Taken from Jennison 1942, modified from Wells 1934)

Diameter of Droplet in μ .	Evaporation Time in Seconds
2,000	515 0
1,000	129 0
500	32 0
200	5 2
100	1 3
50	0 31
25	0 08
12	0 02

being dependent for their transportation on air currents. It has been shown experimentally that micro-organisms attached to droplet nuclei are rapidly dispersed throughout rooms and even buildings, and are inhaled by anyone breathing air containing them (Wells and Wells 1936).

Trillat (1938) described an interesting experiment in which he atomized one litre of a fluid suspension of *Chromobacterium prodigiosum* in a central court of the Pasteur Institute in Paris. A hundred agar plates were exposed for more than 100 metres around. Every single plate became infected, even those placed to windward of the court.

Droplets are also expelled, though in much smaller numbers, during coughing, as in a sneeze, the vast majority are not projected more than 2 or 3 feet. In talking, particularly in the pronunciation of sharp consonants like p, b, t, f, k and s, droplets are often expelled through the teeth; their average size is larger than of those in a sneeze, but their initial velocity is lower, and few travel more than a foot. (For photographs of droplet expulsion in a sneeze, see Weyrauch and Rzymkowski 1938, Bourdillon and Lidwell 1941, Jennison 1942.)

What proportion of droplets and droplet nuclei contain bacteria, and of these what proportion are infective, probably varies greatly from patient to patient. Duguid (1946a), who held plates 3 inches in front of the mouth of patients during a series of six voluntarily produced coughs, found that 39 out of 87 patients suffering from scarlet fever or carrying hæmolytic streptococci in the throat expelled infected droplets. The number of infected droplets varied with individual patients from 0 to 400. Of all droplets expelled by the 87 patients, only 10 per cent. contained hæmolytic streptococci. Similar observations were made on patients affected with diphtheria and pulmonary tuberculosis.

Droplet nuclei are, of course, much smaller than droplets and hence less often contain bacteria; on the other hand there are far more of them.

From experiments carried out on patients during sneezing and coughing, Duguid (1946b) found that droplets and droplet nuclei ranged from 0.5 to 2,000 μ in diameter, 95 per cent. being between 2 and 100 μ . Of the droplet nuclei proper 97 per cent. were between 0.5 and 12 μ , the commonest being 1-2 μ . Assuming that saliva, which provides most of the

Growth Accessory Substances or Bacterial Vitamins

stances; most strains of dysentery bacilli and *Proteus* require only nicotinic acid. These nutritive requirements are generally believed to be an expression of limitations in the synthetic abilities of the microorganisms. The assumption

B COMPLEX VITAMINS REQUIRED BY VARIOUS SPECIES OF BACTERIA*

Bacterium	Substance Required in Micrograms per ml of Culture						
	Thiamine	Riboflavin	Pantothenic Acid	Nicotinic Acid	Biotin	Pyridoxine	Folic Acid
<i>Staphylococcus aureus</i>	0.003			0.20			
<i>Streptococcus hemolyticus</i> C203S	0.001	0.004	1.00	0.10		2.00	
<i>Streptococcus epidemicus</i> X40		0.10	0.50				
<i>Streptococcus hemolyticus</i> D-NYS			1.25				
<i>Streptococcus zymogenes</i>						0.50	0.0005
<i>Streptococcus lactis</i> R				1.20			
<i>Pneumococci</i>					0.10		
<i>Shigella dysenteriae</i>				0.20			
<i>Proteus morganii</i>							
<i>Brucella melitensis</i>	0.02			0.03	0.10	0.0001	0.06
<i>Lactobacillus casei</i>			0.04				0.00005
<i>Lactobacillus arabinosus</i> 17-5					0.10	0.00015	
<i>Clostridium tetani</i>				0.05			
<i>Corynebacterium diphtheriae</i>					1.00		0.0002

* Data from various authors, modified from table of Stokes, Guinness and Foster. Jour. Bact., 1944, 47:293.

being the same as that first postulated for the essential amino acids, viz., the substance in question is an essential metabolite which cannot be synthesized by the microorganism and therefore must be supplied as such. From this point of view there appear to be varying degrees of limitation. For example, the influenza bacillus requires the entire coenzyme molecule but most bacteria

which measured volumes of air were drawn through a funnel and allowed to play on agar plates. One of the simplest and most effective devices for the collection and counting of air-borne bacteria is Bourdillon's slit sampler (Bourdillon *et al.* 1941). In its original form air was sucked through a slit 0.25 mm wide and impinged on the surface of a rotating agar plate immediately beneath. The rate of suction was adjusted to 1 cubic foot of air per minute, and samples of 2-10 cu. ft. were collected depending on the bacterial content of the atmosphere. The improved model of slit sampler (Report 1948) is now used almost exclusively in Great Britain for measuring bacterial contamination of the air.

Instead of arranging for the organisms to impinge on solid surfaces, they may be collected in broth and the bacterial content of the broth determined by plating. An apparatus in which measured volumes of air are bubbled through broth in a flask was described by Wheeler, Foley and Jones (1941) and is sometimes referred to as the bead-bubbler device. Other methods, such as the atomizer-bubbler device and the aeroscope, making use of the same principle, are referred to in a paper by DuBuy, Hollaender and Lackey (1945), who made a comparative study of various sampling devices for air-borne organisms (see also Report 1948). As a rule counts obtained by the impingement method are less than those obtained by bubbler devices, because many of the colonies are derived from groups of bacteria and not from single organisms. Passage through broth tends to disintegrate the bacterial clumps, though to a very variable degree, and to increase the number of units from which colonies may develop. For estimating the particle-size distribution of viable air-borne bacteria the method described by Goldberg (1950) may prove useful.

Ellott (1941) devised an ingenious method for enumerating bacteria in the air, but so far it has never been properly developed.

Its essential features are first, the trapping of suspended particles by drawing the air sample through a water-vapour mist, and second, the condensation of water on any particles left in suspension by cooling the saturated air. This latter principle is derived from the Wilson cloud chamber, in which the path of electrons is made visible by depositing on them water droplets condensed from a saturated atmosphere by a sudden reduction in pressure.

An electrostatic sampler, depending on the deposition of bacteria according to their electrical charge, was described by Luckiesh, Taylor and Holladay (1946).

For estimating the degree of nasopharyngeal pollution of the air, Gordon (1902-03) long ago, in his study of the ventilation of the House of Commons, suggested that *Str. viridans* should be used, in much the same way as *Bact. coli* is used for measuring the excretal pollution of water. This suggestion was revived by American workers (Buchbinder *et al.* 1938, Wells, Wells and Mudd 1939). Williams and Hirsch (1950) found that the best method was to collect the bacteria in a slit sampler on 5 per cent serum agar containing 5 per cent. sucrose, 1/100,000 crystal violet and 1/400,000 potassium tellurite. The growth of most staphylococci and micrococci was inhibited on this medium, *Str. salivarius* formed distinctive mucoid colonies, and other streptococci could be identified by suitable sampling of colonies. *Str. viridans* is also useful for testing the efficacy of bactericidal agents designed to destroy potentially pathogenic micro-organisms in the air. It can be sprayed into a closed room, and the disinfection rate estimated by plate counts on air collected at measured intervals. The sampling of dust for hæmolytic streptococci is described by Williams (1949b).

media, and cultivation of a chromogenic organism in the presence of dilute antiseptics may result in the complete suppression of pigment formation. Similarly, the incubation of *B. prodigiosum* at 37° C. results in cultures which entirely lack the brilliant red color characteristic of the organism. As a rule oxygen is indispensable to pigment production and most chromogenic species yield no trace of pigment when grown under anaerobic conditions. *Spirillum rubrum*, however, which grows well in the presence of oxygen, is said to form its red pigment only in oxygen-free media. In the case of some of the chromogens the presence of certain chemical compounds or elements in the medium is essential to, or greatly favors, pigment production. Thus phosphates and sulfates have been found necessary for the production of pyocyanin by *Ps. pyocyaneus*, and sodium tartrate has been shown to favor the production of pigment by *B. prodigiosum*. Carbohydrate media (potato, rice and wheat starch) often lead to a particularly brilliant chromogenesis.

The bacterial pigments are chemically of diverse nature. Many of the red and yellow pigments are insoluble in water, but soluble in alcohol, ether and chloroform, and appear to be lipochromes, a group of fatty pigments widely distributed throughout the plant and animal kingdoms; others, like the fluorescent pigment, are soluble in water, but not in ether or strong alcohol, and may be related to the anthracyanins. Of the bacterial pigments, the structure of pyocyanin is known; it has been found to be an entirely new type of dye and the first instance of a phenazine derivative occurring in nature. The bacteriochlorin found in the purple and green sulfur bacteria appears to be closely related to chlorophyll *a*.

The relation of many of the bacterial pigments to the physiology of the organisms is uncertain. Bacteriochlorin is, of course, responsible for the photosynthetic activity of the purple and green sulfur bacteria. Cytochromes *a*, *b* and *c* are present in all bacteria except the obligate anaerobes, separately and in various combinations, and presumably function as a part of the respiratory mechanisms operative during aerobic growth. The presence of cytochrome, however, does not impart color to the bacteria. Other pigments such as pyocyanin and phthiocol (a yellow pigment of the tubercle bacillus) are reversibly oxidized and reduced and may function as hydrogen transport systems; there is some evidence that the former may function as an alternative hydrogen transport system to heme systems in azide poisoning.⁹⁷ The majority of bacterial pigments, however, fall into the group of lipochromes or carotinoids, and appear to be physiologically inert.

Toxins. The results of the parenteral inoculation of bacteria free culture filtrates into experimental animals must be interpreted with considerable caution. A great variety of proteins, bacterial and otherwise, yield, on hydrolysis, split products of high molecular weight that are toxic, and the complex amines that may accumulate in the cultures of certain bacteria likewise give rise to marked symptoms, or even death, when injected intravenously. Some bacteria, however, have been shown to form what are called true toxins to distinguish them from the non-specific toxicity of the decomposing material present in the cultures of a variety of bacteria. These toxins diffuse out of the cells and may be found in the culture fluid and are, in consequence, also spoken of as "soluble toxins" or "exotoxins." They are apparently protein in nature, large molecules

⁹⁷ Lichstein and Soule: Jour. Bact., 1944, 47:239.

(1941a), who studied the air flora in canteens and hospital wards over a period of some months, found that the result of oil treatment of wooden floors was to reduce the number of organisms dispersed into the air on sweeping by about 80 per cent. The oil can be applied to wooden or to linoleum floors, but not to rubber, cement, or concrete. A fresh application has to be made every 6 or 8 weeks.

Observations by van den Ende and Spooner (1941), however, showed that, if bacterial contamination of the air in hospital wards was to be adequately controlled, treatment of the floor alone was insufficient. The bedclothes, particularly the blankets, which constituted the source of the dust, had to be treated too. It proved possible to do this by soaking them in 30 per cent. liquid paraffin in "white spirit," followed by removal of the excess in a hydro-extractor (see van den Ende, Edward and Lush 1941). Woollen materials retained about 2.5 per cent. of their weight of oil, and cotton materials about 6.5 per cent.; they were not perceptibly oily to the touch. In this way the heavy contamination of the air that normally accompanies bedmaking was avoided. Further work by van den Ende and Thomas (1941) showed that liquid paraffin in white spirit could be replaced by technical white oil emulsified very finely in water by means of suitable wetting agents, which had the advantage of imparting bactericidal activity to the bedclothes. The use of these oil-in-water emulsions resulted in a 99 per cent. reduction in the number of organisms liberated during bedmaking. (For technique of laundering garments with oil, see Harwood *et al.* 1944, Puck *et al.* 1946, Leslie 1953.) In practice, Wright, Cruickshank and Gunn (1944) found that in order to control streptococcal cross-infection in measles wards it was essential to oil not only the floors but also the bedclothes, sleeping garments, towels and similar articles. When this was done, a substantial reduction in the cross-infection rate and in the middle-ear complication rate was effected. The experience, however, of most other workers in wards and barracks has not been so encouraging. There is general agreement that the thorough oiling of floors and bedclothes lowers both the general and the hæmolytic streptococcal content of the air considerably, but that it has little effect in diminishing the respiratory or the wound cross-infection rate (Report 1946, Begg *et al.* 1947, Shechmeister and Greenspan 1947, Rountree 1947). The conclusion has in fact been drawn that epidemic respiratory disease is spread more by droplets and droplet nuclei than by dust, though further observations will be required before this can be accepted.

Handkerchiefs are often heavily contaminated with bacteria. Dumbell, Lovelock and Lowbury (1948) found that by gentle shaking about 15,000 particles could be liberated into the air. As the average diameter of these particles was $20\ \mu$, they were able to contain many bacteria. What part the handkerchief plays in the spread of infection is difficult to determine, but investigations in progress with the use of medicated handkerchiefs do not suggest that it is great.

Droplet-borne Infection.—It is clear that no method of chemical disinfection of the air can control droplet-borne infection. Even good ventilation cannot prevent the direct passage of droplets from one person to another. Mechanical means alone, such as face masks worn either by the patient or by the exposed persons or by both, afford hope of interfering with the direct exchange of the respiratory flora. As demonstrated by actual photographs, masks made of fabric alone are not completely impermeable to the droplets expelled during sneezing;

Bacterial Activity in Mixed Cultures

which are synthesized by the organisms capable of forming them, and they have many properties in common with enzymes. The ability to form toxins is one that is possessed by relatively few bacteria, the best known being diphtheria and tetanus bacilli, the botulinus bacillus and the organism of symptomatic anthrax. These substances are, by a wide margin, the most potent poisons known. There is no explanation for this pronounced toxicity, for the soluble toxins do not differ obviously from other plant and animal proteins. Some of the pathogenic bacteria synthesize other substances termed hemolysins which bring about the dissolution of red blood cells. These substances also appear to be proteins. The cell substance of some bacteria, such as the cholera vibrio, some of the dysentery bacilli and others, is toxic upon injection presumably because of the presence of intracellular or endotoxins. The endotoxins differ in a number of ways from the true toxins, and there is some question as to whether they represent toxic substances synthesized by the bacteria or whether the toxicity of the bacterial cells is a result of the decomposition of bacterial toxins is discussed at length elsewhere (Chapter 8).

The Physical Effects of Bacterial Growth. Both heat and light may be generated by bacterial growth. It has been pointed out earlier that bacteria use only a portion of the energy made available by the oxidation of organic compounds, the remainder being liberated as heat. When circumstances are such that the heat cannot escape as fast as it is generated, the temperature of the bacterial environment rises. The rise may be considerable, the heating of manure piles and damp hay in which bacterial decomposition is proceeding actively may bring them to temperatures as high as 70° to 75° C. Under such conditions the thermophilic bacteria become active and maintain these high temperatures. It has been suggested that spontaneous combustion may result from bacterial activity, but this seems improbable.

The phosphorescence sometimes observed upon decaying fish and meat is found most commonly, though by no means exclusively, in sea water and upon the bodies of marine animals, and a considerable number of species have been described. Sodium chloride and magnesium chloride favor the growth of the phosphorescent bacteria, and one or the other of these salts is essential to the production of light. Luminescence is apparent only in the presence of oxygen, broth cultures from which dissolved oxygen has been exhausted by the respiratory activity of the organisms may be made to glow by shaking. Bio luminescence in certain crustacea, such as Cypodina, results from the oxidation of a substance, luciferin, by molecular oxygen in the presence of an enzyme, luciferase. Presumably a similar mechanism accounts for the production of light by the luminous bacteria, but neither luciferin nor luciferase has been isolated from these organisms.²²

BACTERIAL ACTIVITY IN MIXED CULTURES (BACTERIAL ASSOCIATION)

Although the relatively precise knowledge which has made possible the development of bacteriology as a science has been gained through the study of bacteria in pure culture, it must not be forgotten that in nature a pure

²² Hanes, *Living Light* Princeton University Press, Princeton, 1940. Also, *Ann. Rev. Biochem.*, 1941, 10:531

in contrast to chemical air disinfectants, ultraviolet radiation is most effective at low relative humidities (Report 1948).

2. *Disinfectant Sprays and Vapours.*—In the early days of antiseptic surgery Lister introduced a carbolic spray to sterilize the air over the wound. It is doubtful how far it was effective. Hewlett (1939), for example, found that to kill typhoid bacilli on paper slips, it was necessary to vaporize 35 gm. of phenol per 1,000 cu. ft. of air, and even then an hour's exposure had to be allowed. It is probable that, had the bacilli been atomized, a lower concentration of phenol for a shorter time would have proved effective; but since more powerful germicides than phenol are now known, the interest of the carbolic spray is mainly historical.

Though sulphurous acid and formalin vapours were used for terminal disinfection in isolation hospitals and sick-rooms, little progress in the destruction of bacteria in the air of occupied rooms appears to have been made till Douglas, Hill and Smith (1928) showed that it was possible to bring about the rapid death of *Bact. coli* in the atmosphere by means of a sodium hypochlorite solution sprayed into the air in very high dilution. In spite of this impressive result, no general interest in the possibilities of aerial disinfection was aroused till the publication of Wells's papers in 1934 and 1936 on the nature and significance of air-borne bacteria in the causation of respiratory disease. In 1938 appeared Trillat's paper on bacterial aerosols, and Masterman's paper on the purification of the air of inhabited rooms by a hypochlorite spray. Since then, intensive study has been devoted by several workers to the problem of finding a suitable chemical agent for disinfection of the atmosphere. Some of the more promising substances may be referred to here.

(a) *Sodium hypochlorite* Masterman (1938, 1941) found that a reduction of over 99 per cent in the bacterial content of the atmosphere could be brought about by atomizing 1 gm. of a 1 per cent. sodium hypochlorite solution in 40,000,000 ml. of air. Evidence suggests that, so far as bacteria of nasopharyngeal origin are concerned, such a high percentage reduction could hardly be expected.

Andrewes (1940) states that 5 ml. of a 1 per cent. hypochlorite solution were required to kill rapidly 95 per cent. of streptococci suspended in the air of a room of about 1,000 cu. ft. capacity; this gives a concentration of only 1 in 5.7 million. Pulvertaft (1944) made similar observations. Bourdillon, Lidwell and Lovelock (1942) found that 2.1 ml. of 1 per cent. sodium hypochlorite solution per 1,000 cu. ft., i.e. 1 in 13.5 million, killed almost all the bacteria projected into the air by sneezing in 3 to 4 minutes. Challinor (1943) observed a reduction of about 33 per cent. of the aerial bacteria, as measured by the slit sampler, after spraying a room with 1 per cent. hypochlorite solution in the proportion of 11 ml. to 1,000 cu. ft., i.e. a concentration of only 1 in 2.6 million. Edward and Lidwell (1943), using hypochlorous acid gas, found it necessary to use an initial concentration of 1 in 800,000 to destroy 90 per cent. of the bacteria. Nevertheless, it is clear that sodium action on most non-sporing pathogenic bacteria.

The effective concentration of sodium hypochlorite is influenced by a number of factors. A low relative humidity and a high content of organic matter in the air are both unfavourable. So also is the presence of phenol vapour. HOCl attacks metal, and is decomposed by organic matter, so that it should not be used, nor should the solution be used on rubber or wool. Hypochlorite sprays are voided in certain electrical equipment, such as transformers.

culture is the exception rather than the rule. Actively decomposing organic matter, for example, contains a great number of bacterial species, and a diseased animal may, and usually does, harbor a variety of microorganisms. The responsibility for a given phenomenon has, in many cases, been fixed upon but one kind of bacterium present in the naturally occurring mixture, the remainder having no apparent significance. The great majority of infectious diseases are phenomena of this type, and it is possible, therefore, to speak of a given bacterium as the causal or etiologic agent of such a disease. Similarly, one microorganism may be shown to be responsible for one kind of fermentation while another takes place as the result of the activity of a quite different bacterium. Modern bacteriology clearly rests upon the generalization that may be made from these facts, the concept of specific microbial etiology. But is this the whole story? It may be asked, first, whether the microorganisms present in a mixed culture are passive with respect to one another or whether one species is affected, either adversely or favorably, by proximity to other actively metabolizing forms; and, second, whether or not a given phenomenon for which no single species can be shown to be responsible may result from the combined activities of a heterogeneous group of bacteria.

Beneficial Associations. With regard to the first of these questions, bacteria have been found to exhibit the various degrees of relationship known to exist among the higher forms of life. Examples of *symbiosis*, a relation in which there is mutual benefit, are rare among the bacteria, but *metabiosis* or *commensalism*, in which one member benefits while the other is unaffected, is commonly observed. Perhaps the most obvious example is the growth of an obligate anaerobe in mixed culture with an aerobic organism. The latter uses up the oxygen in the immediate vicinity, thereby allowing the anaerobe to develop. Sporulating anaerobes are sometimes carried in culture with a non-spore-forming aerobe, for subsequent separation by heat is readily accomplished. In the breakdown of cellulose in the soil, the preliminary hydrolysis by the cellulose-decomposing bacteria yields glucose which may be utilized by a variety of organisms present which are unable to bring about the initial hydrolysis. Similarly, the hydrolysis of protein material by proteolytic bacteria liberates amino acids which may be further decomposed by non-proteolytic forms.

Bacterial Antagonisms.⁹⁹ Bacterial antagonism or antibiosis is also of common occurrence. Fermentative and proteolytic types of bacteria generally do not prosper equally well in mixed culture. As pointed out previously, the bacterial proteases are for the most part tryptic in nature and work best in alkaline environments. The acid reaction resulting from the fermentation of carbohydrate is distinctly inhibitory to these organisms, a fact which has been the basis of attempts to replace the proteolytic flora of the large intestine with an aciduric flora consisting of organisms such as *Lactobacillus acidophilus*. The formation of ptomaines or complex amines, whose absorption presumably leads to "autointoxication," by the proteolytic organisms is thereby suppressed or prevented. Numerous other examples of bacterial antagonism have been reported, many of them apparently somewhat more specific than acid inhibition. The well known overgrowth of the diphtheria bacillus by *Staphylococcus*

⁹⁹ Waksman *Microbial Antagonisms and Antibiotic Substances*. Commonwealth Fund, New York. Revised edition, 1947.

from these findings. Observations carried out in dormitories of barracks for naval recruits over a period of 6 months showed that saturation of the air with triethylene glycol, though greatly reducing the number of air-borne bacteria, was ineffective in preventing influenzal, streptococcal or other acute respiratory infections (Report 1952). Experience of the use of this substance in infants' wards at the Johns Hopkins Hospital was likewise disappointing (Loosli *et al.* 1947).

(d) *Other Substances*.—Lovell, Lidwell and Raymond (1944) suggested the use of *hydroxy-acids* as aerial disinfectants, and claimed considerable success for lactic acid. This substance can be vaporized by dropping a solution on to a hot plate, and the vapour proves bactericidal in a concentration of 3.5 mgm. per cubic metre. *Ozone* in concentrations tolerable by the human respiratory tract has little or no bactericidal activity under practical conditions (Elford and van den Ende 1942, Ingram and Haines 1949). *Balsamic smokes* were investigated by Twort and Baker (1910). They noted that smoke given off by smouldering cardboard, tobacco, and incense was germicidal. Incense was the most effective; 1 part in 160 million of air destroyed salivary organisms within 15 minutes.

The mode of action of the vaporized disinfectants is subject to controversy. According to Trillat (1938), Pulvertaft and Walker (1939), and Twort and his colleagues (1940), atomized disinfectants exert their action in the form of minute droplets—hence the term aerosol coined by Trillat—which come into contact with the suspended bacteria. Other workers maintain, on the contrary, that they act as simple gaseous disinfectants. Masterman (1941), for example, points out that minute droplets of the size postulated by Twort, namely $0.4\text{--}1.0\ \mu$ in diameter, would evaporate in a fraction of a second (see p. 2273), and would therefore no longer be in the aerosol or mist form. He brought evidence to suggest that the disinfectant action of sodium hypochlorite was due to gaseous hypochlorous acid set free by the carbon dioxide in the air. Similarly there is reason to believe that propylene glycol acts essentially in the gaseous form. Vaporization of this substance by heat is just as effective as spraying it in the form of a mist (see Robertson, Bigg, Puck, Miller and Baker 1942). Moreover, calculation shows that the maximum number of contacts possible between the disinfectant and the bacterial droplets is insufficient to account for the extreme rapidity of sterilization so long as the disinfectant is present in the form of an aerosol. If, however, the aerosol droplets are assumed to evaporate almost at once, then sufficient molecules of gas will be liberated within 2 or 3 seconds to produce a lethal concentration of propylene glycol in the bacteria-containing droplets. This explanation is also in accord with the observation that, in the form of a liquid, propylene glycol has a very low bactericidal action, whereas in vaporized form it is among the most active disinfectants known (see also Puck 1947). The term aerosol is somewhat misleading and should probably be reserved for minute droplets of oily substances that do not evaporate in the atmosphere. For a discussion of the theoretical considerations underlying aerial disinfection, the reader is referred to papers by Puck (1947) and Nash (1951) and Report (1948).

It may be mentioned that both to ultraviolet light and to disinfectant vapours Gram-negative bacilli are more susceptible than Gram-positive cocci; that organisms of whatever nature are harder to kill when dried on to particles of dust than when present in the form of droplet nuclei; and that a varying proportion

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The Antibiotic Substances.¹⁰² Investigation of antagonistic micro organisms has shown that in many instances the antagonistic effect is due to the activity of a substance formed by the antagonist which is toxic for the affected bacterium. These substances have been termed *antibiotic substances* or *antibiotics* by Waksman. Such substances have been known for many years, the first, pyocyanin, having been isolated by extraction of "blue pus" in 1860 before the causative bacterium, *Pseudomonas pyocyaneus*, was discovered. Many others are now known. In general they are more effective on gram-positive bacteria, and differ from one another with respect to their relative toxicity for various species of bacteria. Their chemical properties indicate that they are of diverse nature, including polypeptides, sulfur-containing compounds, lipids, pigments, quinones and organic bases. Some have been prepared in crystalline form and in a few cases the structure is known. These substances have become of greatly increased interest in recent years since it has been found that, while some are highly toxic to man, others are effective chemotherapeutic agents.

Antibiotic Substances from Bacteria. PYOCYANIN AND PYOCYANASE. The best known antibiotic substances of bacterial origin are those produced by *Pseudomonas pyocyaneus* and by aerobic sporulating bacteria of the genus *Bacillus*. *Ps. pyocyaneus* forms two substances having antibiotic activity, the chloroform-soluble phenazine compound *pyocyanin*, and a substance known as *pyocyanase*, which appears to be a lipid, whose activity is associated with the presence of unsaturated fatty acids in the molecule. A third substance which also shows activity may be isolated by ether extraction; this is a yellow pigment, *hemipyocyanin*, and a derivative of pyocyanin. These substances are all relatively toxic to higher animals.

TYROTHRINICIN. An alcohol-soluble, water-insoluble polypeptide having antibiotic activity, which he named tyrothricin, was isolated by Dubos¹⁰³ from a gram-positive aerobic sporulating bacterium, *Bacillus brevis*. Further investigation made possible its separation into two components, *gramicidin* and *tyrocidin*, of somewhat different properties though both contain "unnatural" amino acids of the *d* series. Gramicidin is a large cyclopeptide containing relatively large amounts of tryptophane and is effective only on gram-positive bacteria. A very similar substance, *gramicidin S*, is formed by a thermophilic variety of *B. brevis* which differs in that it has considerable activity against gram-negative bacteria and is a cyclopeptide hydrochloride with one free amino group, no free carboxyl and one hydrochloride residue, made up of one residue each of *l*-ornithine, *l*-proline, *l*-valine, *l*-leucine and *d*-phenylalanine. Tyrocidin is active against both gram-positive and gram-negative bacteria *in vitro*, but its activity is almost completely inhibited by serum proteins. These antibiotics are surface-active substances and their antibacterial activity is perhaps attributable to their destructive effect on the cell wall of the bacterium.

BACILLUS SUBTILIS ANTIBIOTICS. A number of antibiotic substances have been isolated from strains of *B. subtilis* which vary somewhat in their properties and antibiotic activity, though all appear to be polypeptide in nature. *Subtilin* is effective chiefly on gram-positive bacteria and certain of the acid-fast

¹⁰² See Benedict and Langlykke: Ann. Rev. Microbiol., 1947, 1:193, Bailey and Cavallito: *ibid.*, 1948, 2:143

¹⁰³ Dubos: Jour. Exp. Med., 1939, 70:1.

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The Antibiotic Substances

Bacilli It has low toxicity and is effective in the therapy of experimental infection of mice with pneumococci and guinea pigs with anthrax, and may prove to be an effective chemotherapeutic drug. *Bacitracin* is similar to subtilin and effective on gram-positive bacteria. It is somewhat toxic but non-irritating to tissue and has possibilities as a chemotherapeutic agent. *Bacillin* is highly active on both gram-positive and gram-negative bacteria *in vitro* and is only moderately toxic, but, like tyrocidin, its activity is destroyed by blood and it is completely ineffective as a chemotherapeutic agent. *Eumycin* does not affect gram-negative bacteria but has *in vitro* activity against the diphtheria and tubercle bacilli and some of the fungi. Its toxicity is low but its therapeutic efficiency is not known. *Licheniformis* resembles subtilin in its properties and is regarded by some workers as identical with it.

Other antibiotics from bacteria include *colicin* or *colistatin* referred to above, which are formed by coliform bacteria and inhibit gram-negative bacilli such as the dysentery bacilli. Some of the bacterial pigments show antibiotic activity, including *violacein* from *Chromobacter violaceum*, *phthiocol* formed by the tubercle bacillus, and *iodinin* from *Chromobacter iodinum*, discussed later in connection with competitive inhibition. A substance named *diplococcin* has been obtained from milk streptococci which is active against species of *Bacillus*, *Clostridium*, *Lactobacillus* and *Streptococcus* and which has considerable chemotherapeutic activity in experimental streptococcus infections. None of these have been fully studied.

Antibiotic Substances from Actinomycetes A number of antibiotic substances have been isolated from actinomycetes, especially by Waksman and his co-workers. The more important of these may be considered briefly. The first to be isolated were *actinomycin A* and *actinomycin B*, isolated from cultures of *Actinomyces antibioticus*.¹⁰¹ The two substances, soluble in organic solvents, were both prepared in crystalline form, and the former found to be markedly bacteriostatic and weakly bactericidal, while the latter was chiefly bactericidal. Both are highly toxic to higher animals. *Actinomycin A* is a red pigment and appears to be a polycyclic nitrogen compound, it is usually referred to simply as *actinomycin*. The substances *actinomycin* and *actinomycin* *lysosyme* are formed by *A. albus* and *A. violaceus* respectively and differ from other antibiotics in that their action is primarily lytic. The former is a thermolabile protein which is precipitated by alcohol, acetone and ammonium sulfate, and the latter is a water soluble, thermostable substance very similar to egg white *lysozyme* (avidin) but not identical with it.

STREPTOTRICIN This antibiotic was isolated from cultures of *A. lavendulae* by Waksman and his co-workers. It is adsorbed on charcoal and may be eluted with mineral acids. It is thermostable and precipitated from aqueous solution by alcohol. It is an organic base and has been prepared as the crystalline reineckate which contains no methoxy, methyl or hydrolyzable acetyl groups, and little is known of its structure. It is more active on gram-negative than on gram-positive bacteria, is bactericidal as well as bacteriostatic, and has considerable fungistatic and fungicidal activity. It is of very low toxicity and has therapeutic promise but has not been tested extensively.

¹⁰¹ Waksman and Woodruff. *Jour. Bact.*, 1941, 42: 231. Waksman and Trischler. *Jour. Biol. Chem.*, 1942, 142: 519.

CHAPTER 92

THE BACTERIOLOGY OF WATER, SHELL-FISH, AND SEWAGE

WATER

Bacterial Flora in Water.

It is convenient to divide the bacteria found in water into three groups:

A. NATURAL WATER BACTERIA.

In this group are included those organisms that are commonly found in waters free from gross pollution. They may be subdivided as follows:

Bacilli.

- | | |
|-----------------|--|
| (1) Fluorescent | { Gelatin liquefied. <i>Ps. fluorescens-liquefaciens</i> . |
| | { Gelatin not liquefied. <i>Ps. fluorescens-non-liquefaciens</i> . |
| (2) Chromogenic | { Red pigment. <i>Chr. prodigiosum</i> , <i>Chr. indicum</i> . |
| | { Orange " <i>Chr. aurescens</i> . |
| | { Yellow " <i>Chr. ochraceum</i> . |
| | { Violet " <i>Chr. violaceum</i> . |

- (3) Non-chromogenic { These organisms belong mainly to the *Achromobacter* group. They are sometimes divided up according to their reactions on gelatin and milk; but they have so far not received sufficient study to render their classification possible. Gram-positive, spore-bearing aerobes, which produce acid and gas in lactose, appear to be not uncommon (Greer 1923, Porter *et al.* 1935). Members of the coli-typhoid group are not natural inhabitants of entirely unpolluted waters.

Cocci.

- (1) Chromogenic. Generally yellow pigment formed.
(2) Non-chromogenic. *M. candicans*, *M. aquatilis*, *M. coronatus*.

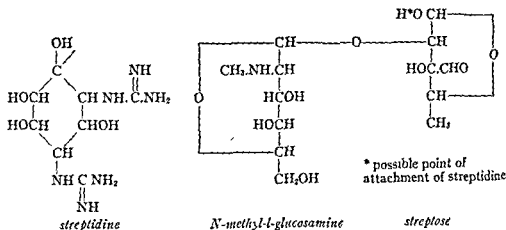
Sarcinae.

Chiefly *Sarcina lutea*.

B. SOIL BACTERIA.

These organisms, though not normally inhabitants of water, are frequently washed into it during heavy rains. Most of them belong to the group of aerobic spore-bearing bacilli, such as *B. subtilis*, *B. megaterium*, and *B. mycoides*. Others, such as *Bact. aerogenes* and *Bact. cloacae*, which may be found on grain, plants and decaying vegetation, and which are aerobic non-sporing bacilli. By ch

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¹⁰⁶ See the review of streptomycin therapy by Keefer *et al.*: *Jour. Amer. Med. Assn.*, 1946, 132:70.

the greatest numbers of organisms in rain are found during the dusty months of June, July, August, and September. Montsouris is situated on the outskirts of Paris; in the centre of the city the air was found to contain 6,040 bacteria per cubic metre, and the rain 19,000 bacteria per litre.

TABLE 185

	Bacteria per cubic metre of Air at Montsouris. Average for 10 Years.	Bacteria per litre of Rain Water at Montsouris. Average for 2 Years.
January	160	8,000
February	145	1,320
March	225	2,920
April	310	2,140
May	305	2,440
June	355	5,600
July	465	5,600
August	455	8,300
September	310	5,770
October	190	3,220
November	195	3,250
December	165	4,330
Mean	275	Mean 5,300

In the open country the organisms may not exceed 10 or 20 organisms per litre of rain.

(b) *Snow*.—This tends to be less pure than rain, probably because the snow-flakes have a greater surface on which to collect suspended particles in the atmosphere; and also because their low temperature conduces to the survival of bacteria. In snow situated on the tops of high mountains, where it will be remembered that Pasteur found the air to be practically sterile, there are hardly any organisms.

(c) *Hail*.—Curiously enough, hail contains more bacteria than either rain or snow. Belli (1902) examined hail that fell in Padua during July, 1901, and found no fewer than 140,000 organisms per litre. Examination of the bacteria showed that they belonged to nine different types. During the formation of hail, it seems probable that rapidly ascending currents of air carry the raindrops up into a region of the atmosphere where they are solidified; falling down they are melted, and again swept upwards and frozen. After they have been frozen and thawed a number of times the hailstones are thrown out on the periphery of the storm centre and finally come to earth (Mason 1902). It is suggested that the air currents carry up to the cloud region quantities of dust, which is thus incorporated in the hail. It is difficult to explain otherwise the presence in it of vegetable cells, and of fluorescent and soil bacteria.

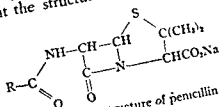
(d) *Ice*.—The number of organisms in ice depends on the nature of the water from which the ice is formed. With the exception of the ice of glaciers, it is generally impure. Its low temperature is favourable to the survival of most bacteria; hence the self-purification that occurs in waters on storage occurs hardly at all, or very slowly, in ice. For a short review on the bacteriology of ice, see Jensen (1943).

(e) *Shallow Wells*.—If protected from contamination in the immediate vicinity

The Antibiotic Substances

Antibiotic Substances from Molds PENICILLIN. Of the antibiotic substances produced by molds, penicillin, found in cultures of *Penicillium notatum* and by far the best known. This is largely because it is highly bacteriostatic and almost completely non-toxic for higher animals and has been found to be a highly effective chemotherapeutic agent. It was first found by Fleming in 1929 but its possibilities were not appreciated until the work of Oxford chemists in 1940. Penicillin is readily soluble in water and is extracted from acidified aqueous solution with ether or amyl acetate, and may then be taken out of the organic solvent by shaking with dilute bicarbonate or barium hydroxide solution. It may be purified by chromatographic adsorption and the salts are stable in the dry state.

Very considerable interest has attached to its structure because of its high chemotherapeutic activity. It has been prepared in crystalline form; there is some uncertainty about the structure and the β lactam form shown here is



The β -lactam structure of penicillin

generally regarded somewhat more favorably than the alternative incipient azlactone form.¹⁰⁷ Prior to the elucidation of its structure, it was apparent that penicillin is not a single substance in that the product formed by different strains of *Penicillium* differed. First three fractions, designated F, G, and X in the United States and I, II, and III in Britain, were differentiated, and later a fourth fraction, designated K, was found. The antibiotic produced by *Aspergillus giganteus* is a reduced form of penicillin F, and designated dihydro F, and the antibiotics produced by *Aspergillus flavus* and originally described as flavicidin and flavicin were found to differ from penicillin F only in the position of the double bond in the side chain. With the elucidation of the structure of penicillin it became apparent that these penicillins are derivatives of the parent compound shown here with respect to the R group. Thus, penicillin F is Δ^2 pentenyl penicillin, dihydro F is *n*-amyl penicillin, G is benzyl penicillin, X is *p*-hydroxy benzyl penicillin, and K is *n*-heptyl penicillin. These relationships are summarized in the accompanying table. Of these penicillins, X is the most effective, G and F less so, and K is relatively ineffective, presumably because it is rapidly inactivated in the body.¹⁰⁸

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¹⁰⁸ See the discussion by Housewright, Berkman and Henry. *Jour. Immunol.*, 1947, 57, 343.

from that in surface waters; the organisms develop slowly at room temperature, there are few liquefying colonies, and chromogenic organisms are relatively numerous (Prescott and Winslow 1913).

B. Nutrition.—The amount of available food supply is probably the most important factor of all in determining the number of bacteria in a given water. When organic matter is plentiful, organisms abound; when it is scarce they are few, and tend to die out.

C. Temperature.—The effect of temperature varies with the amount of organic matter present. A rise of temperature in a water containing an ample food supply for the bacteria causes them to multiply rapidly; but when the organic matter is small in quantity, a rise in temperature has the reverse effect; this is probably due to early exhaustion of the food supply, and the consequent diminution in rate of multiplication of the bacteria.

A low temperature, independent of the amount of organic matter present, favours the survival, though not the multiplication, of bacteria. Houston (1913) added typhoid bacilli to raw Thames water, and maintained the samples at

TABLE 186
INFLUENCE OF TEMPERATURE. (Houston 1913.)

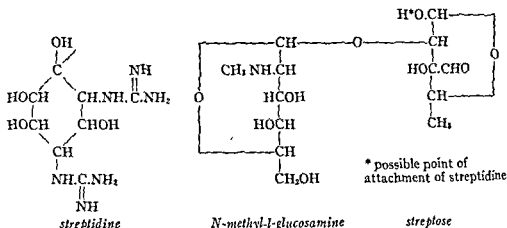
Degrees.	No. of Bacilli per ml. surviving after weeks.								
	1.	2.	3.	4.	5.	6.	7.	8.	9.
0° C. . .	47,766	980	65	34	3	3	2	1	00
5° C. . .	14,894	26	6	3	03	0.1	00	—	—
10° C. . .	69	14	3	03	00	—	—	—	—
18° C. . .	39	3	04	00	—	—	—	—	—
27° C. . .	19	0.1	00	—	—	—	—	—	—
37° C. . .	5	00	—	—	—	—	—	—	—

temperatures varying from 0° C. to 37° C. The initial number added was 103,328 per ml. of water. Table 186 shows how much more rapid was the death of the organisms at 37° C. than at freezing-point. Hamilton (1935), who made observations on the Whangpoo river, found that in the short run from Shanghai to Woosung there was a diminution of 16 per cent. in the colon bacteria during the winter months, and of no less than 97–99 per cent. during the summer months.

D. Light.—It has been asserted that the ultraviolet rays of the sun play an effective part in destroying micro-organisms in water. Procaccini (1893) placed drain water, from which the coarser particles had been removed, in glass cylinders 50 cm. deep, and exposed them for 6 hours to the Italian sun in June. A control cylinder was protected from the light (Table 187). The water in the exposed cylinder was practically sterilized; that in the protected cylinder contained rather more organisms than at the start of the experiment.

It would appear that under laboratory conditions the actinic rays of the sun may exert a bactericidal effect. In nature, however, the conditions are altered. One of the main factors hindering the rays is the opacity of the water, which prevents their penetration for more than a short distance. Even in clear water,

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between the amount of dissolved oxygen in lake water and the bacterial content; but other factors may possibly have obscured the effect.

H. Protozoal Content.—Huntmüller (1905) showed that flagellates contribute notably to the extermination of bacteria in water. River water naturally polluted by bacteria, or suspensions of *Salm. typhi*, could be cleared in 4 days if flagellates—*Bodo saltans* or *Bodo ovatus*—were added (see also Kyriasides 1931). Stokvis and Swellengrebel (1911) demonstrated a similar action by infusoria—*Colpoda cucullus*. The bacterial destruction was preceded by a rise in the number of protozoa in the water, and was probably due to active ingestion, though this was not demonstrated conclusively. Aerobic conditions and a temperature between 10° and 30° C. were essential. König (quoted from Thresh and Beale 1925) found that in 1 ml of water from a well at the Hygienic Institute of Munich 21 million added typhoid bacilli perished in 24 hours. This he attributed to the action of protozoa. In pure water the death-rate in this time was trifling. Increasing attention is being paid to the action of predatory plankton in the self-purification of naturally polluted waters (Butterfield *et al.* 1931, Hoskins 1935). By keeping the bacterial population below the saturation point, it is suggested that the plankton favours the continuous multiplication of bacteria in the water, which results in its turn in a progressive oxidation of organic matter.

I. Rainfall.—The effect of rainfall on the bacterial content of a water is complex. Rain falling after a drought washes large numbers of soil organisms into the water, and hence increases the numbers of bacteria. If the rain continues for some days, fewer organisms may be carried in during the later period of rainfall so that the stream is diluted with water purer than its own; its bacterial content therefore decreases. A pure stream may be contaminated by rain; an impure stream may be benefited by dilution. As a rule, rivers and upland surface waters contain their greatest numbers of bacteria after heavy rainfall. In Lake Windermere Taylor (1910) observed a close association between the periodic fluctuations in bacterial content and the amount of rain that had fallen in the drainage area during the previous week. The effect is ascribable not only to the influx of organisms, but to the addition of nutritive substances, and to the increased oxygenation resulting from the beating effect of the rain upon the surface of the water.

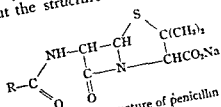
J. Season.—The monthly variation in the bacterial content of waters depends chiefly on the temperature and the rainfall. In this country, the highest counts are generally found in the winter months, when the temperature is low and the rainfall greatest. Rivers show more variation than upland surface waters (Tables 188 and 189). The Thames in winter is swollen by heavy rain, and the quality deteriorates in consequence of the scouring effect over the whole drainage area. In summer many sources of pollution have dried up, and much of the water in the river is virtually stored or filtered water, derived from underground sources of supply; hence the bacterial content falls (Houston 1917). In upland surface waters, a rise in the bacterial content is not infrequent during July and August, the explanation of this is not clear, but it seems not unlikely that it is the result of dust and soil washings carried in by the summer rains (see Table 189).

In countries in which the water supply is augmented by melting snows the bacterial content rises considerably in spring time. Oslo obtains its water supply from a lake about 160 metres above sea level. During most of the year, Schmelck (1888) found that the number of organisms per ml. was 10 to 60, rising

The Antibiotic Substances

Antibiotic Substances from Molds. **PENICILLIN.** Of the antibiotic substances produced by molds, penicillin, found in cultures of *Penicillium notatum* and produced to a lesser extent by certain other penicillia such as *P. chrysogenum*, is by far the best known. This is largely because it is highly bacteriostatic and almost completely non-toxic for higher animals and has been found to be a highly effective chemotherapeutic agent. It was first found by Fleming in 1929 but its possibilities were not appreciated until the work of Oxford chemists in 1940. Penicillin is readily soluble in water and is extracted from acidified aqueous solution with ether or amyl acetate, and may then be taken out of the organic solvent by shaking with dilute bicarbonate or barium hydroxide solution. It may be purified by chromatographic adsorption and the salts are stable in the dry state.

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prevents the excess distribution of a bad supply on any one day. Even in a river, the water may not be homogeneous; samples taken from one side may be different in their bacterial content from those taken near the other side (see Table 191). In a reservoir, whether natural or artificial, a greater degree of homogeneity is attained. (3) Devitalization: the organisms die in large numbers, probably from lack of food supply, and ingestion by protozoa. Houston added cholera vibrios to raw river water, and found that after 1 week's storage their numbers had been reduced by 99.9 per cent.; after 3 weeks they could not be isolated even from 100 ml. of water. Table 190 shows the effect of storage on the London water.

The reduction occurs not only in pathogenic organisms and *Bact. coli*, but in organisms of all sorts, though not always equally. Houston states that even 1 week's storage would be more efficacious, in reducing the initial numbers of typhoid bacilli or cholera vibrios in a water, than sand filtration. It should be remembered that pathogenic organisms usually survive longer in pure than in impure water, and therefore pollution of purified water in reservoirs and water mains should be guarded against very carefully (E. W. Taylor 1949).

TABLE 190
EFFECT OF STORAGE. 1907-08. AVERAGE RESULTS. (Houston 1913.)
Bacteria per ml.

	Gelatin, 20°-22° C.	Agar, 37° C.	No. of Samples with <i>Bact. coli</i> in 0.01 ml.
River Thames before storage	4,465	280	10 1%
River Thames after 15 days' storage at Chel- sea	208	44	1.1%
Reduction	95.3%	84.3%	89 1%

It is probably owing to storage that lake waters are so much purer than the streams that feed them. Some rivers with a very low gradient may offer conditions suitable for sedimentation.

L. Filtration.—Natural filtration occurs on a large scale, resulting in the accumulation of the underground deposits of water that are tapped by deep wells and main springs. Its efficacy in the removal of bacteria depends on the nature of the soil, and the depth of the strata penetrated. In loose, porous soils a greater depth must be traversed to ensure the same degree of purification that is attained by filtration through a more compact soil. Evidence suggests that, in a soil of moderate density, the greater part of the bacteria are removed in the first 10 or 15 feet. This is the reason why deep well water is so pure.



Artificially, sand filtration is used to remove bacteria from water in order to render it potable. Houston (1913) finds that this process, which in the London water follows storage for 30 days, removes 98 per cent. of the residual bacteria.

M. Other Factors.—It is known that certain rivers have a bactericidal effect on some of the intestinal organisms. Thus Arloing and Sempé (1924) state that the water of the Saône inhibits the growth of *Bact. coli* but not of *Salm. typhi*. The water of the Isère inhibits the growth of *Salm. paratyphi A*, the Rhône *Salm. typhi*; and the sea at Havre *Shigella dysenteriae* (Arloing and Chavanne 1925,

to divide, and it is generally believed that some process or processes of cell division are affected. There is, however, no evidence as yet as to the nature of its action with respect to the physiology of the bacterium. It is inactivated by an enzyme, penicillinase, which is produced by a wide variety of microorganisms.¹⁰⁹ It is a highly effective chemotherapeutic agent, especially in staphylococcus and streptococcus infections and certain other diseases such as gonorrhea and syphilis.

Standardization. The antibiotic activity of penicillin is assayed by inhibition of growth of standard strains of staphylococci. Growth may be measured by the turbidity of developing broth cultures, but the most commonly used method is that developed by the Oxford group and makes use of inhibition of

THE NATURALLY OCCURRING PENICILLINS

Name	Synonym	Source	R
penicillin F	penicillin I	<i>P. notatum</i> <i>P. chrysogenum</i>	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2^-$
dihydro F penicillin	gigantic acid	<i>A. giganteus</i>	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2^-$
flavicin flavacidin	F type	<i>A. flavus</i>	$\text{CH}_3\text{CH}=\text{CHCH}_2\text{CH}_2^-$
penicillin G	penicillin II	<i>P. notatum</i> <i>P. chrysogenum</i>	 CH_2^-
penicillin X	penicillin III	<i>P. notatum</i> <i>P. chrysogenum</i>	 CH_2^-
penicillin K	penicillin IV	<i>P. notatum</i> <i>P. chrysogenum</i>	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2^-$

growth on agar media. It consists of the inoculation of an agar plate so that a uniform film of growth will develop; small cylinders of glass or porcelain are set end down on the inoculated surface. One is filled with standard penicillin solution which contains one *Oxford Unit* per milliliter, and the others with varying dilutions of the unknown. After incubation there is a clear zone of growth inhibition around the cylinders, about 24 millimeters in diameter in the case of that containing the standard solution. The *Oxford Unit* is, then, defined as "that amount of penicillin which when dissolved in 1 ml. of water gives the same inhibition (i.e., area) as this standard." An International Unit has now been adopted¹¹⁰ which is defined as the activity of 0.6 microgram of the pure crystalline sodium salt of penicillin II or G, a quantity of which is available as a standard.¹¹¹ This unit closely approximates the *Oxford Unit*.

¹⁰⁹ See the discussion by Woodruff and Foster: *Jour. Bact.*, 1945, 49:7.

¹¹⁰ League of Nations, *Bull. Health Organization*, 1945-46, 12:181.

¹¹¹ The method of assay used by the Food and Drug Administration is given by Hunter and Randall: *Jour. Assn. Offic. Agr. Chem.*, 1944, 27:430.

Public Health Association (1936*b*) in the United States have each described standard methods for its performance, to which reference should be made by those desirous of further knowledge. Great attention has to be paid to the sampling of the water (for details see Report 1939*a*), and to the technical performance of the various procedures involved (for error of these see Wilson *et al.* 1935). For reference to the methods used in France see Buttiaux (1951), in the Argentine see Ferramola (1947), and for general information on the bacteriology of water and water supplies see E. W. Taylor (1949). Changes in the coliform flora of samples stored at different temperatures are considered in Reports (1952, 1953*c*).

Plate Count.—In general, the analysis consists in an enumeration of the organisms or groups of organisms capable of forming colonies on a standard nutrient agar medium incubated aerobically at 20°–22° C. for 3 days or at 37° C. for 2 days. Since not all the organisms in water are viable; since many viable organisms, such as those of the anaerobic and nitrifying groups, do not develop under the particular conditions provided; and since some of the organisms occur in groups which give rise to only a single colony—it is clear that the colony count corresponds not to the total number of organisms or even to the number of viable organisms, but only to the number of bacterial units that are able to multiply under the nutritional, respiratory, and temperature conditions supplied. For this reason it should be reported either as the "number of colonies developing per ml.," or more simply as the "plate count per ml."

Coliform Count.—Besides a general bacterial enumeration, an attempt is made to estimate the number of coliform bacteria in the water, and often to ascertain the proportions of the various types of these organisms. The ideal method of doing this would be a direct plate count on a differential medium. So far, however, no medium has been devised that will distinguish with certainty between coliform and non-coliform organisms, or between the different types of coliform bacilli. Partial success in these respects was claimed by Tonney and Noble (1930, 1931*a, b*, 1932*a, b*) for their ferrocyanide citrate agar medium, by Gehm and Heukelekian (1935) and Olsen (1952) for eosin methylene blue agar, by Littman and Stark (1938) for citrate ricinoleate agar, and by Chapman (1951) for triphenyltetrazolium chloride agar.

The usual technique for estimating coliform bacilli is by the dilution method in a liquid medium, which allows the observation of gas production—an important property serving to distinguish coliform from most non-coliform bacilli. As has already been pointed out (Chapter 4), the dilution method is subject to a big sampling error. The results depend not only on the number of dilutions prepared and on the number of tubes of each dilution inoculated, but also on the number of organisms present in each unit volume of the original water. Hence attempts have been made to devise the most economical method of carrying out the examination consistent with reasonable accuracy (Halvorson and Ziegler 1933*a, b*, Swaroop 1940, 1941). Even greater effort has been expended in endeavouring to convert the results obtained into terms of the probable number of organisms present (see Greenwood and Yule 1917, McCrady 1918, Hoskins 1934, Matuszewski and Supińska 1937, Haldane 1939, Pomeroy 1940, Buchanan-Wollaston 1941). No perfect medium has yet been devised that will enable all coliform organisms to develop and produce gas, while suppressing the growth, or at least the gas formation, of other organisms.

British practice favours MacConkey's bile salt lactose medium, but in the United

The Antibiotic Substances

NOTATIN. A second antibiotic substance produced by *P. notatum* was described independently by British workers as *notatin* and by American workers as *penatin* and *penicillin B*. It differs from penicillin in that it is insoluble in organic solvents and is active only in the presence of glucose. It appears to be a flavoprotein, producing hydrogen peroxide from glucose, and the peroxide is responsible for its antibiotic activity.

In addition to penicillin and notatin, a wide variety of antibiotic substances have been found to be produced by molds. Some of these are:

ASPERGILLIC ACID. This substance is produced by *Aspergillus flavus* and is active against both gram-positive and gram-negative bacteria. It is an amphoteric substance, soluble in alcohol, ether and acetone but not in petroleum ether, and its empirical formula is $C_{12}H_{20}O_2$. It is very toxic for higher animals.

PENICIDIN. This substance is also active against both gram-positive and gram-negative bacteria and is produced by a number of species of *Penicillium*. It is extracted from culture fluid by ether at neutrality and precipitated from ether solution by the addition of petroleum ether as a yellow oil. Little is known of its nature.

PENICILLIC ACID. This substance is responsible for the antibiotic activity of some species of *Penicillium*, notably *P. puberulum* and, in contrast to penicillin, is most effective against gram-negative bacteria. It is water-soluble and has been isolated in colorless crystalline form and found to have the empirical formula $C_8H_{14}O_4$. Two other metabolites of *P. puberulum*, *puberulic acid* and *puberulonic acid*, are also bacteriostatic. The former has been isolated as a colorless, crystalline dibasic acid with the empirical formula $C_8H_6O_6$, and the latter as bright yellow prisms with the formula $C_8H_4O_6$. *Puberulic acid* appears to be a quinonoid and *puberulic acid* the corresponding quinol.

CLAVIFORMIN (CLAVACIN, PATULIN, TERCININ, EXPANSIN). This substance was isolated from *Aspergillus clavatus* and named clavacin, and later from *Penicillium patulum* and called patulin, the two were later found to be identical. The name *tercinin* has been used as a synonym for patulin. It is most active against gram-negative bacteria. It is soluble in ether, chloroform, alcohol and water and has been shown to be anhydro-3-hydroxymethylene-tetrahydro- γ pyrone-2-carboxylic acid. It shows greater bactericidal activity than most of the antibiotic substances.

FUMIGACIN (MILLVOLIC ACID). This substance has been found in cultures of *Aspergillus fumigatus* and is active on gram-positive bacteria. It is soluble in alcohol and sparingly soluble in water and has been isolated in crystalline form.

CITRININ. This substance is formed by *Penicillium citrinum* and acts on both gram positive and gram negative bacteria. It is soluble in water and in alcohol and appears to be an organic base.

FUMIGATIN AND SPINULOSIN. These substances were isolated from cultures of *Aspergillus fumigatus* and *Penicillium spinulosum* respectively. Fumigatin has been found to be 3-hydroxy-4-methoxy-2,5-toluquinone, and spinulosin is 6-hydroxy fumigatin. Fumigatin is the more active substance and is effective against such organisms as *Staphylococcus aureus*, the anthrax bacillus and the cholera vibrio.

citrate is distinctive, in the coliform group, of the intermediate and aerogenes-cloacæ types. By making use of probability tables it is thus possible to obtain within 3 or 4 days a quantitative estimate of the organisms belonging to the coliform, the faecal coli, and the intermediate-aerogenes-cloacæ groups. In this method, though the different types may be present in very unequal proportions in the fermented tubes, transference to selective liquid media will enable growth of even minimal numbers to occur.

The value of the 44° C. MacConkey test for picking out *Bact. coli* of faecal origin has now been abundantly confirmed (see p. 761); though in Madras and Singapore some strains of *aerogenes* type—probably Irregular Type VI (see Wilson *et al.* 1935)—were found capable of producing gas at this temperature (Raghavachari and Iyer 1939a, Boizot 1941). The citrate test is less specific for members of the intermediate-aerogenes-cloacæ group when used for direct inoculation from a fermented 37° C. MacConkey broth culture, owing to the ability of some late-lactose and non-lactose fermenters and even *Bact. coli* to grow in this medium (see Bardsley 1938a, Raven *et al.* 1940, Ferramola 1940). In practice, however, this is not usually a serious drawback, since most false positives in citrate occur with the more polluted waters, which would be condemned in any case on the presumptive coliform or the faecal coli count. The tendency now, however, is to omit the citrate test in routine water analysis and to rely on tests for *Bact. coli* I.

The selective liquid media method was adopted officially by the Ministry of Health (Report 1939a) for the differential coliform test in the bacteriological examination of waters. In the extensive trials during the second world war, it proved to be reliable, and to be economical in both time and medium.

Though C. B. Taylor (1945), working in a particular locality, found that as many as 15 per cent. of *Bact. aerogenes* strains produced gas in MacConkey's medium at 44° C., the specificity of the 44° C. MacConkey test for *Bact. coli* in this country is very high. Occasionally Irregular Type vi, which resembles *Bact. aerogenes*, causes trouble. It is found particularly in jute and hemp, and may multiply under suitable conditions in yarn used for packing joints of water pipes (E. W. Taylor and Whiskin 1945). Again, especially in polluted water that has been chlorinated, *Cl. welchii* may cause confusion. This organism usually forms large quantities of gas at 44° C. but only slight acidity. To overcome the difficulties caused by both these organisms Mackenzie, Taylor and Gilbert (1948) recommend subculturing the presumptive positive 37° C. MacConkey broth tubes into brilliant green bile broth and into peptone water and incubating both at 44° C. overnight. The brilliant green suppresses the growth of *Cl. welchii*, and the negative indole test on the peptone water culture excludes *Bact. coli* I.

Classification of Coliform Organisms in Water.—In Chapter 28 we have discussed the classification of lactose-fermenting coliform bacilli according to the general principles of systematic bacteriology. In water analysis it is of greater advantage to classify these organisms in relation to their habitat, paying particular attention to their excretal or non-excretal origin. Such a classification is given in Table 192.

It will be seen that *Bact. coli* Type I, Irregular Type I, and possibly some of *Bact. coli* Type II (see Bardsley 1938a, Stuart *et al.* 1942) appear to find their natural habitat in the mammalian intestine. It is true that strains belonging to the intermediate-aerogenes-cloacæ (I.A.C.) group are often found in faeces, but as a rule they are present in only very small numbers. Their natural habitat is still unknown (see Taylor 1942a, 1951), but most of the evidence suggests that

CLITOROXIN. This substance is produced by *Gliocladium fibratum* and some species of *Trichoderma* and has also been found admixed with fumigatin in cultures of *A. fumigatus*. It is soluble in chloroform and benzol alcohol and sparingly soluble in water. It has been isolated in crystalline form and found to contain both nitrogen and sulfur and possibly an indol nucleus. It is effective on both gram-positive and gram-negative bacteria but unstable except in acid solution.

CHAETOMIN. This antibiotic is formed by *Chaetomium cochliodes* and occurs for the most part in the mycelium. It is extracted from the mycelium with acetone and from the filtrate with ethyl acetate and purified by shaking with bicarbonate, treatment with petroleum ether, and chromatographic adsorption. It is active against gram-positive bacteria but not against gram-negative bacteria.

Bacterial Synergism.¹¹² Association between bacterial species often affects the organisms in such a way that the ability of the individual species to decompose organic compounds may be modified or extended. This phenomenon, termed bacterial synergism, is sometimes qualified into "antagonistic" and "beneficent" synergism, but since the majority of the changes which indicate the combined work of two or more bacterial species cannot be construed as either favorable or unfavorable to the microorganisms, this distinction is often a difficult one to make. Perhaps the simplest examples of synergism are those in which the enzyme system of one organism supplements that of another. A mixed culture of a streptococcus which ferments lactose to acid without gas, and one of the paratyphoid bacilli which does not ferment this sugar, in lactose broth produces an acid and gas fermentation. Similar results have been obtained with mixed cultures of non-sucrose-fermenting *Bacterium coli* and streptococci fermenting sucrose to acid. In such cases the colon-typoid organisms further oxidize the split products resulting from the decomposition of the disaccharide to gas. Probably similar complementary action is the basis of increased yields of propionic acid in mixed cultures of propionic acid bacteria and lactic acid cocci over that obtained with the propionic acid organism alone. Other decompositions, however, are not so readily explained. Cellulose, for example, is often much more rapidly decomposed by bacteria in mixed culture than it is by component cellulose-decomposing organisms in pure culture.

The course of decomposition may be qualitatively different in mixed culture from that in pure culture. It has been shown, for instance, that the acetone-butyl alcohol fermentation of *Clostridium pectinovorum* is transformed into a lactic acid fermentation by mixed culture with *Bacillus voluntans*. A considerable number of pairs of organisms have been shown to produce gas from carbohydrates in mixed culture when neither alone is able to bring about this change,¹¹³ and similarly, mixed cultures of *Clostridium chauveii* and *Bacillus paralactici* form butyl alcohol from glucose although neither alone could produce this change. This ability of mixed cultures of bacteria to bring about

¹¹² See the review by Burrows: *Synergistic Aspects of Bacterial Populations*. Biol. Symp. 1942, 8:89.

¹¹³ Holman and Meekison; Jour. Inf. Dis., 1926, 39:145.

milk after preliminary heating to 80° C. for 10 minutes to destroy non-sporing organisms, or by use of W. J. Wilson's (1928) glucose sulphite iron agar medium.

Typhoid and paratyphoid bacilli are difficult to demonstrate in water, partly because of technical difficulties, and partly because, unless the contamination of the water is continual, these organisms will have died out by the time suspicion of their presence is aroused. By suitable enrichment and selective media—particularly W. J. Wilson and Blair's (1931) bismuth sulphite brilliant green medium—it is often possible to demonstrate their presence in grossly polluted water, though practically never in water that is only slightly contaminated (Ruys 1940-1). Other organisms of the *Salmonella* group may likewise be found, both in polluted river water and in the intestinal contents of fish living in it (Leiguarda *et al* 1950). The *membrane filter* has been recommended for concentrating pathogenic organisms in water (see Clark *et al*. 1951), but it is doubtful whether it will add to the sensitivity of our present methods (see Broscheit 1953). The same doubt underlies the indirect method proposed by Guelin (1918) of examining the water for bacteriophage to different Vi-phage types of *Salm. typhi*.

In the demonstration of *V. cholerae* advantage may be taken of its rapid growth in alkaline peptone water under aerobic conditions, and of the various differential media available (see Chapters 22 and 63).

Interpretation of the Bacteriological Analysis.—Before attempting to give an opinion on the results of a bacteriological analysis, it is essential to gather particulars of the nature of the water, the method by which it was collected, the time of collection, and the amount of recent rainfall. It is sound practice, though not always possible, for the bacteriologist to make a topographical survey of the gathering ground, so as to ascertain the extent and the kind of pollution to which it is subject. If he is unable to do this personally, he should consult a map on which the source of the water and the immediate environment are indicated. Particular care must be given to the mode of collection of the sample; otherwise contamination, especially with coliform organisms, may disturb the interpretation of the results (for instructions, see Report 1939a).

The whole aim of the bacteriological analysis of water is to find evidence of excretal pollution. Since we can rarely isolate directly specific pathogenic organisms, such as *Salm. typhi*, we resort to estimating first of all the number of living bacteria of all sorts in the water, and secondly the number of living bacteria of intestinal origin. The greater the number of bacteria, the greater, presumably, is the amount of decomposing organic matter. The more bacteria of intestinal origin there are, the more likely are pathogenic species to be amongst them. Our evidence therefore is circumstantial, and is frequently open to doubt in its interpretation.

Unfortunately we cannot lay down absolute standards for all waters; but we can lay down a standard for any one water. This is possible by frequent and regularly repeated examinations, which teach us the range of normal variation. We become acquainted, in fact, with the bacterial character of the water. Any striking deviation from the norm is at once regarded with suspicion. On a water which we examine for the first time and for which we have no absolute standards, it is often difficult to express more than a tentative opinion. A high agar count at 22° C., for example, may be significant or it may not; and though the sanitary survey may assist us on this point, it may not be till several examinations under different conditions have been made that we can offer a definite opinion.

Bacterial Synergism

changes of which none of the component species is capable is of interest in connection with the concept of emergent evolution.

One consequence of bacterial association has been of considerable interest in regard to mixed and secondary infections—the alteration of the virulence of a pathogenic bacterium by association with other microorganisms. This alteration may be either a decrease or an increase in the pathogenic powers of the organism. The mild course of mixed infections of the diphtheria bacillus and Friedlander's bacillus has been referred to above, and in this case decrease in virulence is presumably due to inhibition of toxin formation. It is well known that inoculation with soil containing anthrax spores does not always produce the disease even though pure cultures isolated from the same soil are highly virulent. This apparent decrease in virulence is thought by some to have its explanation in the stimulation of a pathogenic bacterium of the animal by the extraneous bacteria present in the soil. On the other hand, virulence may often be increased by inoculation of a non-pathogenic form, with the bacillus of malignant edema increases the virulence of the latter in mixed culture. The mixture of *Bacterium prodigiosum*, a non-pathogenic form, with the bacillus of experimental animals become fatal. Similarly the virulence of the hemophilic bacteria (Chapter 25) is readily raised by inoculation of the experimental animal with mixed cultures. One of the more interesting examples of the effect of microbial association on virulence is that of swine influenza, in which it has been shown that the disease is produced through the combined action of a bacterium and a filterable virus, neither of which is able to produce the disease by itself. Phenomena such as this suggest that the etiology of bacterial associations and the mechanisms which give rise to the observed results is in an unsatisfactory state. Experimental evidence is fragmentary, not in the sense of paucity of observation—the literature on the subject is a voluminous one—but because of a certain discontinuity which makes generalization difficult if not impossible. The interpretation of experimental results obtained with pure cultures is often difficult, and when mixed cultures of two or more different organisms are used, the difficulties are greatly multiplied. The loose use of terminology (some workers use symbiosis, meta-biosis or commensalism, and synergism synonymously) is no doubt an inevitable consequence of lack of knowledge of many phenomena brought about by bacteria under natural conditions probably lies in the development of knowledge of the association of these organisms with one another. It may be noted in passing that bacteria may live an associative existence with higher animals and plants. Symbiotic nitrogen fixation and the relation of the cellulose-decomposing bacteria to their herbivorous animal hosts are among the more obvious instances. Infectious disease of man is, of course, an example of antibiosis or antagonism.

coli type—is the most delicate index we have of recent excretal pollution. With regard to the importance of the intermediate and aerogenes-cloacæ types (I.A.C.) opinion is divided. There are those who maintain that, because these organisms are found in faeces—though only in small numbers—their presence cannot be neglected. There are those who draw attention to the fact that organisms of the aerogenes type are often present in infected urine (Hill *et al.* 1929, Burke-Gaffney 1933), and may constitute the dominant type of coliform bacilli in human faeces (Parr 1936). There are others who point out that, if recent excretal pollution has occurred, faecal coli will undoubtedly be found too, so that the presence of other types in the absence of faecal coli can generally be ignored.

The interpretation is to some extent affected by the rate at which these different organisms die out after gaining access to water. Here again the available information does not provide us with a clear answer.

The vitality of these organisms in water varies with several factors. Houston (1913) found that storage of water for 15 days reduced the number of *Bact. coli* by 80-90 per cent. Gray (1932) and Burke-Gaffney (1933) bring evidence to show that *Bact. coli* dies out more rapidly than *Bact. aerogenes*. Ruchhoft and his colleagues (1933) find that both organisms disappear at about the same rate. Platt (1935) finds that this rate depends to some extent on the temperature, *Bact. aerogenes* surviving longer than *Bact. coli* at 18°-20° C., but not at 0° C. or 37° C. Raghavachari and Iyer (1939b) suspend their judgment. Hamilton (1935), in China, finds that in the summer months *Bact. aerogenes* may actually multiply in water under favourable conditions.

On the whole, the evidence suggests that organisms of the I.A.C. group tend to be rather more resistant to environmental conditions than faecal coli. Their presence in water, in the absence of faecal coli, may indicate either (1) that non-polluted dust or soil has gained access to the water; or (2) that excretal contamination has occurred at a time sufficiently remote to permit the disappearance of all faecal coli organisms; or (3) that the water is subject to a minor degree of excretal contamination heralded, for some reason which is not yet clear, by the appearance of organisms of the I.A.C. group before true faecal coli; or (4) that a contaminated water has been insufficiently chlorinated; or (5) that the water—and this applies to wells and pumps—is being contaminated from such material as old sacking, leather washers, jute packing, or decaying leaves, in which organisms of the I.A.C. type are actively growing. Which of these explanations is correct, it is usually impossible to say on any one sample of water without further examination.

In this country a high proportion of waters giving a positive presumptive coliform test contain faecal coli (Bardsley 1934, 1938a), and it is therefore often unnecessary to distinguish between the coliform types. In the routine control of water supplies from which coliform bacilli are usually absent, the appearance of these organisms demands immediate attention, and differentiation should be carried out in order to obtain a clue to the probable source of pollution. It is likewise called for when a new supply is under examination, as it is always desirable to learn as much as possible about the flora normally present. In other types of water, differentiation is a matter for individual judgment. Generally speaking, there is little point in determining the nature of the types present in a piped water supply if the presumptive coliform count exceeds about 10 per 100 ml., since even if no faecal coli are found the water will still have to be regarded as probably contaminated. In shallow well waters, however, differentiation may be useful with a coliform count up to about 50 per 100 ml., as the degree of excretal

THE EFFECT OF PHYSICAL AND CHEMICAL AGENTS ON BACTERIA

The separation of the environmental factors, that so profoundly affect the life processes of living cells, into two groups, one designated physical and the other chemical, is undoubtedly artificial in many instances. It is often difficult, if not impossible, to differentiate the physical from the chemical; the lethal effect of a germicidal substance, for example, may be a result of a combination of both physical and chemical activity or its mode of action may lie in the borderland of surface and physical chemistry. Nevertheless, such a separation has a certain reality and is useful for purposes of exposition.

Bacteria, like all other living organisms, consist of protoplasm, a delicately balanced, heterogeneous mixture of various substances in colloidal and true solution. The disturbance of this equilibrium through, for instance, the precipitation of constituent protein, is incompatible with the continuation of the complex phenomena of life, and the cell dies. On the other hand, the environment, when favorable, not only does not destroy this equilibrium but makes possible its continuation through growth and multiplication of the organisms. Bacteria, like other organisms, are creatures of their environment; under favorable conditions they multiply rapidly, and under unfavorable conditions either die or remain dormant in a viable state until another opportunity for growth presents itself. In general, they are much more resistant to unfavorable circumstances than are most higher forms of life. A part of this resistance is, of course, a result of the ability of some bacteria to form spores which are relatively highly resistant, but the vegetative cells are considerably more resistant than are the cells of multicellular organisms.

It is not possible to differentiate sharply between these favorable and unfavorable factors. The congruity of a given environmental factor with the protoplasmic equilibrium that is life, is largely a quantitative rather than a qualitative phenomenon. Although high temperatures destroy bacteria, a certain degree of warmth is essential to their growth. Distilled water is toxic to many microorganisms, yet multiplication takes place only in the presence of adequate amounts of moisture. Even the highly active germicidal chemicals often markedly stimulate the growth of bacteria when present in sufficiently low concentrations.

PHYSICAL AGENTS¹

Temperature Relations. Bacteria as a group will thrive under a relatively wide range of temperature conditions. Some of the more hardy, such as

¹ See the review by Rahn *Bact. Rev.*, 1945, 9 1.

into Class 1; that 80 per cent. should not fall below Class 2; and that the remainder should not fall below Class 3. Chlorinated waters ought uniformly to come into Class 1.

For the student's guidance we give two examples of unpiped and unpurified waters destined for consumption by a small population. Though the deep well and main spring water would be safe for a town supply, the second water we quote would have to be purified before its delivery to a large number of consumers would be justified.

DEEP WELL AND MAIN SPRING WATERS

Plate count, agar 3 days at 22° C.	10-200 per ml.
Plate count, agar 2 days at 37° C.	1-10 per ml.
<i>Bact. coli</i> faecal Type I	Less than 1 per 100 ml.
Faecal streptococci	Less than 1 per 100 ml
<i>Cl. welchii</i>	Less than 1 per 1,000 ml.

SHALLOW WELL, LAND SPRING, AND UPLAND SURFACE WATERS

Plate count, agar 3 days at 22° C.	50-500 per ml.
Plate count, agar 2 days at 37° C.	5-30 per ml.
<i>Bact. coli</i> faecal Type I	Less than 5 per 100 ml.
Faecal streptococci	Less than 5 per 100 ml
<i>Cl. welchii</i>	Less than 5 per 1,000 ml.

A filtered river water should conform to the standard of a good shallow well water. If chlorination has been used in addition, then the coli standard should equal that of a deep well water.

The effect of rainfall on the water should be noted carefully. Speaking generally, the less the water is influenced by this factor the better. Though rain in itself contains few bacteria, it may carry in large numbers of undesirable organisms from the soil. A large increase in the number of organisms, especially if attended by a rise in the coli count, should always be regarded with suspicion.

There is often a tendency for more weight to be placed on the results of the bacteriological examination than is justifiable. If the sanitary survey shows the water to be clearly subject to human excretal pollution, then it is not exaggerating to say that a bacteriological examination is redundant. Even should a reasonably good result be obtained on one sample, there is no doubt that repetition on further samples, taken perhaps after heavy rainfall, will reveal the presence of coliform bacilli in large numbers. Bacteriological examination is a delicate tool suitable for revealing pollution too small in amount or too obscure to be noticeable by the ordinary means of the topographical survey. If the relatively crude method of the survey shows the presence of undoubted contamination, then the delicate methods employed by the bacteriologist are seldom required. It follows that no water exposed to known contamination should ever be reported on favourably by the bacteriologist, regardless of what the laboratory findings may be.

Finally we repeat that judgment on the potability of a water can be given only after a careful weighing of all the evidence available. It must be remembered that the mere absence of evidence indicating faecal pollution does not necessarily indicate that pollution has not taken place. All it does show is that at the time the sample was examined, there was no detectable evidence of pollution. It is partly for this reason that frequent examinations are desirable.

The interpretation of the bacteriological analysis, particularly on a new supply,

Temperature Relations

Bacillus subtilis, will grow throughout the range of 6° to 50° C. Others, such as many of the pathogenic forms, are able to grow over a much narrower range, and some of the more fragile organisms will grow only at body temperature, i.e., 37° C., or very close to this temperature. For all these organisms, however, three temperature limits may be distinguished. There is the minimum or lowest temperature at which a given organism will grow, an optimum or temperature of most luxuriant growth, and a maximum, the highest temperature at which growth can take place. The position of these three points differs greatly among different species of bacteria. In general those organisms whose natural habitat is soil or water have optimum temperatures of 22° to 28° C., while those which, presumably as a result of adaptation to a parasitic mode of existence, cannot survive outside the animal body have an optimum of 37° C.

There are, however, bacteria whose optimum temperatures differ considerably from these. Organisms have been found whose optimum temperatures are from 15° to 20° C., and they have been termed *psychrophiles* or cold-loving organisms. Others having optimum temperatures of 55° to 65° C may be found in the soil and hot springs and are called *thermophiles*. The great majority of bacteria have optimum temperatures which lie between these two extremes and, in this terminology, are designated as *mesophiles*.

The optimum temperature for given species of bacteria is generally considered to be that temperature at which the organisms grow "best." The question arises as to whether "best" refers to the rate of growth or to the maximum population attainable. It has been found¹ that temperatures optimum for growth in terms of rapidity of cell division are not always the same as those optimum for the attainment of maximum numbers of cells per unit volume. A culture grown at a temperature at which cell multiplication is most rapid will not attain as high a peak in numbers as a culture of the same organism growing more slowly at a lower temperature.² Other physiological activities of the cell appear to have optimum temperatures that differ from those which are optimum for multiplication. A given sugar may be fermented to a greater extent, albeit more slowly, when the culture is incubated at a temperature somewhat below that optimum for cell division. Similarly, the anthrax bacillus forms spores most abundantly at 30° to 32° C., while its optimum for vegetative multiplication is 37° C.

The continued growth of bacteria at temperatures somewhat higher than optimum may induce physiological changes of a temporary or permanent character. *Bacterium prodigiosum*, for example, fails to form its characteristic red pigment when incubated at temperatures higher than 30° C., but the change is temporary, for subcultures incubated at lower temperatures form pigment normally regardless of how many transfers have been grown at the higher temperature. The anthrax bacillus, on the other hand, when grown for several transfers at 42° C. loses its ability to form spores and becomes avirulent—a change that appears to be permanent. Incubation or storage of bacterial cultures at temperatures lower than optimum does not result in such qualitative

¹ Graham Smith Jour Hyg., 1920, 19 131

² See, for example, Spicer Jour Bact., 1940, 39 517, Steen and Frazier ibid., 1941, 42 479, 501.

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physiological changes; the metabolic activities of the organisms are slowed down and at temperatures below the minimum for growth the bacteria become dormant.

The mechanisms determining the optimum, minimum and maximum temperatures of bacteria are obscure. In some cases they may be dependent upon other environmental factors. It has been shown, for example, that while many of the thermophilic bacteria are able to grow only at temperatures above 50° C. when in contact with air, they are able under anaerobic conditions to grow at the ordinary incubator temperature (37° C.) or even as low as 34° C. Other studies⁴ indicate that the maximum growth temperatures of bacteria bear a definite relationship to the minimum temperature of destruction of respiratory enzymes.

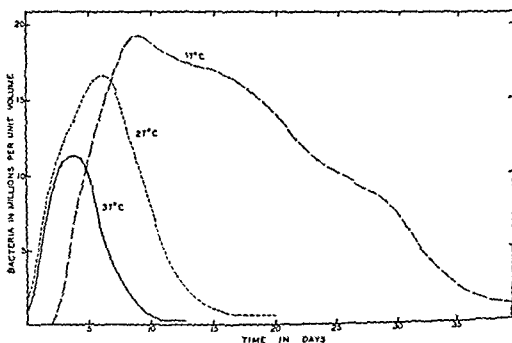


Fig. 22. The effect of different temperatures of incubation on broth cultures of *Staphylococcus aureus*. (After Graham Smith.)

The rate of bacterial growth and metabolic processes increases, like other chemical reactions, with rise in temperature. The temperature coefficient, i.e., the quantitative effect of temperature changes, is such that these rates are roughly doubled for every ten degree rise in temperature. This effect is observed only over a relatively narrow temperature range, from the minimum to the optimum temperature of the bacterial species under consideration.

The Lethal Effects of Heat. Bacteria are readily killed by heat, and the utilization of heat in one form or another is one of the most convenient means of their destruction. The lethal effects of heat are markedly influenced by the amount of moisture present, and so-called moist heat is a much more effective killing agent than dry heat. The resistance of bacteria to moist heat differs somewhat from species to species, the pathogenic forms being in general somewhat less resistant. The resistance of a given species is influenced by

⁴ Edwards and Rettger. Jour. Bact., 1937, 34:489.

difference in the bacterial quality of the water at the inlet and outlet of the bath (Report 1953*b*). In practice the committee recommended that, in swimming-bath water containing 0.2-0.5 p.p.m. of free chlorine, no sample should contain coliform organisms in 100 ml.; and that in 75 per cent. of the samples the plate count at 37° C. on 1 ml. of water should not exceed 10 colonies and in the remainder it should not exceed 100 colonies.

In natural waters used for bathing, such as rivers, lakes and sea, it is impracticable to lay down any bacteriological standard of purity; but where there is reason to believe that sewage is gaining access to the water a count of *Bact. coli* I exceeding 1000 per 100 ml. may be regarded as indicative of potential risk to the bathers (see Moore 1951).

Water-borne Disease.—The main diseases carried by water are enteric fever, dysentery, and cholera. For a description of these reference should be made to Chapters 63, 69, 70. Occasional outbreaks of Weil's disease, tularæmia, infectious hepatitis, and poliomyelitis are on record, but the evidence supporting their water-borne nature is not always convincing. In this country the incidence of water-borne disease is low compared with some parts of the Continent and with the United States, but is by no means negligible. Between 1911 and 1937 there occurred in England and Wales at least 21 outbreaks of disease conveyed by public water supplies, resulting in 1,237 cases of enteric fever, 2,800 of bacillary dysentery, and 7,139 of gastro-enteritis (Report 1939*b*). In the United States, Gorman and Wolman (1939) were able to collect records of 399 outbreaks during the period 1920 to 1936, affecting 115,615 persons. Among the cases of disease were 12,585 of typhoid fever and 101,603 of diarrhoea. Ehassen and Cummings (1948), who analysed the records in the United States for the years 1938-45 inclusive, found a total of 327 outbreaks affecting 111,320 persons. It may be noted that paratyphoid fevers are seldom water-borne. Franklin and Halliday (1937) report one outbreak of paratyphoid A fever in Canada due to contamination of a well water from a resident who had returned to her home suffering from an undiagnosed disease; and there have been 2 or 3 small outbreaks of paratyphoid B fever in this country (see Report 1942, Page 1942). As is apparent from the records just given, diarrhoea and gastro-enteritis figure prominently in water-borne disease. Their nature and causation are not always clear. Some outbreaks may be due to undetected dysentery bacilli, but there seems little doubt that the access of sewage in considerable amount to a water supply may be followed by gastro-enteritis in the absence of any recognized pathogenic organisms (see Kathe and Königshaus 1932, Report 1936*a*, 1937. Pharris *et al* 1938, Ehassen and Cummings 1948, Ross and Gillespie 1952). I

forms of (lene poisoning following the painting of a storage tank.

Infection of the water may occur at the source, during storage, or during distribution. The prevention of water-borne disease demands scrupulous care and supervision of every part of the system, including the protection of gathering grounds (Report 1948) and the health of operatives closely associated with the water itself (see Report 1939*b*).

The danger of bathing in polluted water is difficult to assess. Cases of typhoid and paratyphoid fever due to this cause are invariably sporadic (see Martin 1947), and other sources cannot always be excluded. Unless contamination is recent and heavy, the risk does not seem to be great. If the maximum already suggested for

The Lethal Effects of Heat

two important factors, the ability of the organisms to form spores and the previous history of the culture. Spores are always much more resistant than vegetative forms. Some species when in the spore stage can withstand the temperature of boiling water for upward of sixteen hours. The vegetative forms of most bacteria, on the other hand, are killed at 55° to 58° C. by ten minutes' exposure in the presence of moisture. In general the heat tolerance of bacteria appears to be directly related to maximum growth temperatures.⁵ The time and temperature of incubation influence, to some extent, the heat resistance of vegetative cells. Actively growing cultures in the logarithmic phase are generally somewhat less resistant than are cells removed from cultures containing maximum numbers of viable organisms. The temperature of incubation appears to affect thermal resistance somewhat, in that cultures grown at and above the optimum temperature are more resistant than cultures grown at sub-optimal temperatures.⁶ Previous treatment of both spores and vegetative cells with sub-lethal doses of ultraviolet light reduces their thermal resistance. However, such changes in resistance are usually of no great magnitude.

Of considerably greater quantitative importance is the pH of the liquid in which the organisms are suspended. Variations from neutrality increase, to a marked degree, the heat resistance of many organisms, especially acidophiles, such as tomatoes, and certain bacteria. Acid foods, which have a neutral reaction. Likewise, instruments are boiled increases the time required for sterilization. time tends to reduce rusting.

The death of an organism from heat is determined not only by the temperature reached but also by the time of exposure. The tubercle bacillus, for example, is killed by thirty minutes' exposure at 58° C., twenty minutes at 59° C. and two minutes at 65° C. One may, therefore, determine the thermal death point of a given bacterium by exposure to varied degrees of heat for a constant time; or, similarly, a thermal death time may be determined by holding the temperature constant and varying the time of exposure. Both are useful, the latter possibly somewhat more so.

The effect of heat seems to be injurious even when bacteria are not killed, since the cells that have been heated appear to require a longer period of germination. Hershey⁷ has shown that exposure to sublethal heat prolongs the latent period preceding cell division without affecting the rate of regeneration of the respiratory function, with no evidence of a period of "recovery" distinct from growth. On the other hand, sublethal heating of bacterial spores often has the effect of accelerating germination.⁸

The application of moist heat to the destruction of bacteria may take several forms. Sterilization by means of steam under pressure is the most efficient of these, because it makes possible temperatures higher than 100° C. in the presence of moisture. At fifteen pounds' pressure, for example, the temperature will be 121.3° C., at twenty pounds, 126.2° C., etc. As a rule, exposure to 120° C. for fifteen minutes suffices for the complete destruction of both vegetative

⁵ Cf. Lamanna *Jour. Bact.*, 1942, 44 29.

⁶ Cf. Eliker and Frasier *Jour. Bact.*, 1938, 36 83.

⁷ Hershey *Jour. Bact.*, 1939, 38 563.

⁸ Cf. Frans and Curtan *Jour. Bact.*, 1943, 46 513.

avoids the danger of using the presumptive coliform test. As Dodgson (1938) showed, the 37° test may be grossly misleading, because organisms of the aerogenes-cloacae type may under favourable conditions multiply enormously in mussels, barnacles, raw sea water, and sterilized tank water. By the Clegg and Sherwood method the following standard of interpretation may be tentatively accepted:

<i>Bact. coli</i> per ml of flesh.	Interpretation
Up to 5	Satisfactory
6-15	Suspicious
16 and over	Unsatisfactory

These categories correspond roughly to those adopted by the Fishmongers' Company based on the examination of 0.2 ml. quantities of fluid from 10 individual shell-fish, namely 80-100 per cent. clean, 70 per cent., and 60 per cent. or less (see Knott 1951)

Just as with water, so in the control of shell-fish a careful topographical survey to exclude possible sources of pollution is desirable. Bacteriological examination must remain as an ancillary method to check the results of the sanitary investigation. Valuable information on the sanitary control of the shell-fish industry will be found in a report (1916a) of the United States Public Health Service.

SEWAGE

In this country crude sewage contains about 10 million organisms per ml. capable of developing on gelatin at 20° C., and from 1 to 5 million per ml. on agar at 37° C. In America the numbers appear to be lower. There is often a considerable rise in the summer months. The organisms making up these numbers are of many different kinds, and vary from one sewage to another. Prominent amongst them are the *Proteus* group, the coliform bacilli, streptococci, anaerobic spore-bearing bacilli, natural water bacteria, and the denitrifying bacilli. *Bact. coli* may number 100,000 per ml., *Streptococcus faecalis* 1,000 to 10,000 per ml., and *Cl. welchii* 100 to 1,000 per ml. Pathogenic organisms of the typhoid, paratyphoid, and food-poisoning groups can often be found in crude sewage by the use of appropriate selective media. The poliomyelitis virus has also been demonstrated in it (Paul and Trask 1911, 1912). Sewage is an excellent source of a large variety of bacteriophages.

The process of purification is accompanied not so much by a diminution in the numbers of organisms, as by a change in the distribution of different organisms. Thus in the septic tank, the anaerobic liquefying bacteria are prominent; on the contact beds the aerobic liquefying and the denitrifying bacteria gain the upper hand. It is evident that enormous numbers of bacteria must perish in the process; by a comparison of the microscopic and the gelatin counts, Winslow (1905) found that the ratio of the total to viable organisms was about 20 to 1 in crude sewage, 40 to 1 in effluent, and 100 to 1 in sand filter effluent.

It will be seen that no striking fall occurs in the number of living organisms in the sand filter stage is reached.

A method of sewage disposal that is now widely used is the Activated Sludge Process. Briefly this consists in treating the sewage with about 15 per cent. of bacterially active liquid sludge, in the presence of an ample supply of atmospheric

tive and spore forms of bacteria, although rarely highly resistant spores may be found that are not killed by this exposure. Boiling, i.e., exposure to 100° C., suffices to kill all vegetative forms of bacteria within a few minutes; but sterilization is not effected, for the spores of many of the saprophytic bacilli are not destroyed by boiling for many hours. Since very few of the pathogenic bacteria form spores, boiling contaminated water for a few minutes renders it safe for drinking purposes and the boiling of surgical instruments generally suffices to kill the vegetative cells of pathogenic bacteria, but not the spores of spore-forming pathogens such as those of the tetanus and gaseous gangrene bacilli.

Temperatures as high as 100° C. are not necessary for the destruction of vegetative forms of bacteria, since most of them are killed at 55 to 58° C. if exposed for ten to thirty minutes. In the preparation of vaccines, suspensions of bacteria are usually heated to 60° C. for thirty minutes to one hour (to allow an adequate margin of safety). The tubercle bacillus, *Brucella abortus* and other pathogenic organisms occurring in milk are killed by the process of pasteurization, i.e., heating to 142° to 145° F. for thirty minutes.

Dry heat is much less effective as a germicide than moist heat. Temperatures of 160° to 170° C. must be maintained for two to three hours in order to ensure complete destruction.

The destructive effect of high temperatures on bacteria is apparently associated with the coagulation of constituent protein. The rate of heat coagulation of protein in solution closely parallels the rate of destruction of bacteria by hot water. Furthermore, the effect of the water content of egg albumin on the temperatures necessary for coagulation is similar to the observed relative efficiency of moist and dry heat in the killing of bacteria.⁹

Cold. Bacteria are much less sensitive to low than to high temperatures. As the minimum temperature of a given species is approached and passed, the metabolic activities of the organism become increasingly slower until a state approaching dormancy is reached. Preservation of bacterial cultures in the refrigerator is a common practice, for the rate of death of most bacteria is greatly reduced at low temperatures.¹⁰ Some mortality results from freezing, it is thought to be due to mechanical grinding coincident with the formation of ice crystals. The evidence for this is, however, not altogether indubitable, and attempts to disrupt bacterial cells by alternate freezing and thawing are not particularly successful. These organisms show a remarkable resistance to extreme cold exposure of organisms like the typhoid and diphtheria bacilli to liquid air (−190° C.) and to liquid hydrogen (−250° C.) does not destroy their vitality. Haines¹¹ has shown that the temperature at which frozen bacteria are stored significantly influences the death rate. When *Bacterium coli* was kept at −20° C. 25 per cent of the organisms were still alive after 163 days, while at −2° C. death occurred rapidly and only 4 per cent were found viable after eleven days. Death under such circumstances may possibly be a

⁹ Egg albumin in aqueous solution is coagulated at 56° C.; with 25 per cent water content at 74–80° C., with 18 per cent water content at 80–90° C.; with 6 per cent water content at 145° C. Anhydrous egg albumin may be heated to 170° C. without coagulation.

¹⁰ Some species of bacteria, however, are particularly sensitive to cold gonococci and meningococci, for example, die out much more rapidly in the refrigerator than in the incubator.

¹¹ Haines. Proc Roy Soc, Ser. B, 1938, 124:451.

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Light and Other Radiations

result of the denaturation of constituent protein. The viability of pathogenic bacteria in ice from contaminated sources is discussed elsewhere (p. 252).

Drying. The vegetative forms of most bacteria are killed by drying in air, although the different species exhibit pronounced differences in their resistance. The tubercle bacillus is one of the more resistant and the cholera vibrio one of the more sensitive to drying. In general, the capsulated organisms are more resistant than the non-capsulated forms. Spores are quite resistant to drying, the spores of the anthrax bacillus, for example, will germinate after remaining in a dry condition for ten years or more. The resistance of the pathogenic forms causing disease of the upper respiratory tract is of particular interest in connection with air-borne infection (p. 231), for the length of time that a droplet remains infective is a result, primarily, of the resistance of the particular micro-organism to drying.

The resistance of a given organism to drying is apparently determined to some extent by the rapidity of the drying process and the temperature at which the organisms are dried and stored. If bacteria are frozen rapidly, as with dry ice and alcohol or one of the glycols, dried from the frozen state (the lyophilic process) and the container or ampoule evacuated and sealed, they remain viable with antigenicity and virulence unimpaired for months and years when stored in the refrigerator.¹² It is preferable that they be suspended in a protein-containing solution such as dilute serum before freezing, in the writer's experience broth cultures frequently do not survive. Even bacteria which are sensitive to both cold and drying may be preserved intact by this method.

Light and Other Radiations.¹³ The biological effects of electromagnetic radiation of wave lengths greater than 3000 Å are slight and such effects as may be produced by short radio waves and the visible spectrum are largely due to heat. Visible light becomes bactericidal, however, with the incorporation of appropriate dyes in the suspending medium—photodynamic sensitization—which promote the absorption of light. The pronounced bactericidal activity of sunlight is due in very large part to its content of ultraviolet light of wave length less than 3000 Å. The wave length 2536 Å shows the highest bactericidal activity and it is perhaps more than coincidence that it is also the wave length at which maximum absorption by nucleic acid compounds occurs. Ultraviolet light is non-ionizing but produces an excitation, i.e., the absorption and dissipation of energy do not result in the ejection of an electron and thus the creation of an ion, but rather raise a constituent electron to a higher stage of energy. In contrast to ionizing radiation, ultraviolet absorption depends on molecular rather than atomic structure, and the excited atoms are distributed at random with no tendency to occur in localized areas. This last probably accounts in part at least for the observation that ultraviolet light is much less effective in killing bacteria than is ionizing radiation.

The ionizing radiations include x-rays, α rays, β rays, γ rays, protons and neutrons. X-radiation is, like ultraviolet light, an electromagnetic radiation but the waves are so short, 0.05 to 10 Å, that there is little or no resemblance in the effects produced. Alpha, β , and γ radiations are emitted by radioactive

¹² Floudoff and Mudd. *Jour. Immunol.*, 1935, 29, 389.

¹³ See the general reviews by Lea. *Brit. Med. Bull.*, 1946, 4, 24, *Actions of Radiations on Living Cells*. University Press, Cambridge, 1947.

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substances. Of these, α rays are nuclei of helium atoms and may also be obtained from the cyclotron, β rays and cathode rays are fast-moving electrons, the latter being artificially accelerated; and γ rays are natural x-rays of short wave length. Protons are hydrogen nuclei moving at high speed; they are not emitted by radioactive substances but are obtainable from the cyclotron. Neutrons, also from the cyclotron, do not ionize directly; fast neutrons project hydrogen nuclei already present in the irradiated material as protons, and when a slow neutron is captured by an atomic nucleus, the nucleus emits an α particle, a proton, an electron, or a γ ray, depending on the element. As ionizing radiation passes through a substance it collides with successive atoms, ejecting electrons from them and leaving a trail of ions behind it. The ejected electrons may have sufficient energy to produce additional ionizations as they collide with other atoms, giving rise to clusters of secondary ionization. When these are small they do not add appreciably to the effect of the primary ionization, and the path of the ionizing radiation takes the form of a column of ionization. Occasionally the ejected electron has sufficient energy to travel an appreciable distance and produce a large number of ionizations in a different path; such secondary electrons are called δ rays and may be of considerable importance, especially in irradiation with α rays. The effect of ionizing radiation, then, is the production of columnar zones of intense ionization in the protoplasm of the irradiated cell.

The bactericidal activity of radiation is due to a direct effect on the protoplasm rather than to the production of toxic substances in the surrounding medium. Such an indirect effect, however, may assume importance under some conditions, *i. e.*, when the nutrient medium in which the organisms are to be grown is irradiated as in the irradiation of bacteria inoculated on the surface of an agar medium. Basically, the bactericidal effect is due to chemical changes in the protoplasm but the precise manner in which it is produced is not completely clear. It seems unlikely that it is a result of the toxicity of irradiated protoplasm, for the magnitude of the chemical changes produced by a lethal dose is not great, and it is generally believed that the lethal effect is due to a direct hit of key substances within the cell, *the target theory*, and their destruction by the zone of intense ionization created. The lethal dose of ionizing radiation is directly related to ion density, or number of ions formed, and for *Bact. coli* has been found to range from 4000 r ($r =$ roentgen, a unit of measurement) for β rays to 24,000 r for α rays. This dose of α rays, for example, corresponds to an average of one α particle traversing each $0.06\mu^2$ of cell substance. The lethal effect of the activation produced by ultraviolet is much less per unit of energy absorbed, and about one hundred times as much energy is dissipated in a bacterium killed by ultraviolet light as in a bacterium killed by x-rays. Wyckoff¹⁴ has found that about 4 million quanta are required to kill a colon bacillus with ultraviolet light.

The lethal dose of either ultraviolet light or ionizing radiation is independent of the intensity of the radiation and of the temperature at which irradiation is carried out, and the survival curves are logarithmic. These suggest that the lethal effect is a single unit action by a quantum of ultraviolet

¹⁴ Wyckoff: Jour. Gen. Physiol., 1932, 15:351.

dysentery, or food-poisoning bacilli. The milker constitutes a further source of danger if he is carrying hæmolytic streptococci or diphtheria bacilli in his throat or nose, since these organisms may gain access to the milk *via* the cough spray.

Apart from initial contamination of the milk, imperfect cooling is often responsible for the presence of large numbers of bacteria in any given sample. However carefully milk is produced, sooner or later, provided it is kept at a suitable temperature, it will go sour or putrid as the result of bacterial multiplication. If it is to remain sweet for more than a few hours, all milk should be cooled immediately after production to a temperature of 10° C. or below.

It may be noted that the keeping quality of the milk is determined partly by the degree of initial contamination and partly by the temperature at which it is kept. Milk produced under really cleanly conditions has a considerable bacteriostatic power, and shows little bacterial multiplication for several hours, even when incubated at a favourable temperature. On the other hand, the bacteriostatic effect of milk produced under dirty conditions is very slight, and bacterial multiplication sets in rapidly. For this reason it is much easier to distinguish between a milk produced under sanitary, and one produced under insanitary, conditions if the examination is delayed till the milks have stood at a temperature of 60° F. or so for 12-18 hours. After this time a milk produced under clean conditions will still have a low bacterial count, whereas a milk produced under dirty conditions will contain a large bacterial population.

The production of clean milk is largely a matter of technique, not of structural equipment or refinement. Provided all utensils are sterilized by steam, and the surface of the udder and the milker's hands are cleansed, it is possible to produce milk with a very low bacterial content even under unfavourable conditions. It is, however, not easy to maintain a satisfactory technique day in and day out unless suitable conditions and appliances are provided for the workers.

Types of Bacteria in Milk

The bacteria present in milk from healthy cows may be classified into the following groups:

1. Acid-forming Bacteria.—These organisms ferment the lactose in the milk with the production of acids, mainly lactic acid, which combine with the calcium caseinogenate, liberating free caseinogen; this, being insoluble in water, is precipitated in the form of a smooth, gelatinous curd, which shows little tendency to contract and squeeze out fluid. Lactic streptococci, including *Str. lactis* and *Str. faecalis* (see Chapter 24), are among the commonest members of this group. The closely associated lactobacilli may also be included, though under ordinary conditions they do not appear to proliferate very actively. Staphylococci derived from the udder are not infrequently present in milk, but unlike the lactic streptococci they do not as a rule multiply extensively in raw milk kept at ordinary temperatures. Most of the acid-producing organisms gain access to milk from contaminated utensils, many of them are said to be ultimately of vegetable origin, being found on corn, peas, beans, and cabbage (Stark and Sherman 1935).

2. Gas-forming Bacteria.—These organisms ferment lactose with the production of both acid and gas. They produce a smooth, gelatinous curd, which is often riddled with gas bubbles. The acid produced is largely acetic, which imparts an unpleasant flavour to the milk. Coliform bacilli are among the commonest

Other Physical Agents

light or the zone of intense ionization produced by a single ionizing particle, on a key substance in the bacterial cell. In the light of available evidence, the key system affected appears to be the hereditary or nuclear apparatus. Sublethal irradiation induces changes in bacteria analogous to radiation-induced mutations in higher organisms such as *Drosophila*. X-ray induced mutants of bacteria have been studied by a number of workers¹⁵ and, in fact, as yet it is only among x-ray induced mutants of a single parent strain that the gene recombination referred to elsewhere (p. 184) has been found to occur.

Lethal doses of radiation produce no immediately visible change in bacteria, and they continue to be motile, to metabolize, etc. They are, however, unable to reproduce, cell division does not occur and bizarre forms may develop as a result of continuation of metabolic processes other than some key mechanism of division.¹⁶ The manifestation of the lethal effect at the first cell division following irradiation strongly suggests that some mechanism essential to proliferation has been destroyed, and what would be termed a lethal mutation in higher organisms has been produced.

In general, bacteria are much more resistant to the effects of irradiation than are other organisms, and the lethal dose may be as high as 100,000 r in some instances as compared to approximately 175 r for the guinea pig and perhaps 800 r for the rat. Simple killing, therefore, does not account for the accelerated healing observed following irradiation of certain infectious processes, and the bacterial toxins show only partial inactivation following massive doses of radiation.

Other Physical Agents. *Sonic and supersonic vibrations* apparently destroy vegetative cells upon exposure for sufficient lengths of time. Audible sound waves vibrating at 8900 cycles per second have been found to reduce the numbers of viable organisms in a suspension by 99 per cent in a period of forty to sixty minutes. Ultrasonic waves of 300,000 to 1,000,000 cycles per second have been found to reduce the numbers of viable *Bact. coli* from 70,000,000 to 20,000 per milliliter in fifteen minutes. The destruction is presumably purely mechanical if the effects of rising temperature have been controlled. Many attempts have been made to kill bacteria by passing an electric current through their suspensions. If these organisms could be killed in this manner, the sterilization of fluids such as milk would be a simple matter. It appears, however, that an electric current *per se* has no effect on the organisms, although the generation of heat and the liberation of chlorine (arising from the decomposition of chlorides) incidental to the passage of the current may kill the organisms. At pH's compatible with life, bacteria are negatively charged with respect to water and respond to an electric field by moving to the anode. The migration of these organisms in an electric field is termed *electrophoresis*. Studies on alterations and reversal of charge have shed some light on the mechanism of the agglutination reaction (p. 301). Increased pressures have little or no effect on bacteria unless the

¹⁵ Drea, *Amer. Rev. Tuberc.*, 1938, 38:205; Haberman and Ellsworth, *Jour. Bact.*, 1940, 40:483; Lincoln and Gowen, *Genetics*, 1942, 27:441; Demerec, *Proc. Nat. Acad. Sci.*, 1946, 32:36; Lederberg, *Genetics*, 1947, 32:505.

¹⁶ For electron micrograph studies of irradiated cells see Eisenstark and Clark, *Science*, 1947, 105:553.

cow vary from animal to animal and from day to day, but they may apparently reach several hundred or even thousand per ml. (see Pullinger 1934). Tubercle bacilli may possibly be excreted in the milk in the absence of any definite disease of the udder (Report 1909), but some degree of tuberculous mastitis can generally be demonstrated, though not always clinically, in cows passing infected milk. Milk must become infected from time to time with tubercle bacilli of human type coughed out by tuberculous farm hands or dairy workers, but their demonstration can practically never be accomplished because of the very small number in which they are present and because of their failure to multiply in milk.

Br. abortus.—This organism is responsible for contagious abortion of cattle, which is probably economically more important in this country than tuberculosis. The udder is frequently infected, even in animals that have not aborted (see Chapter 75), though no lesions can be detected by clinical examination. The bacilli are excreted regularly or intermittently in the milk. Their numbers are usually greatest at the beginning of lactation, when they may reach as many as 200,000 per ml, but in the later stages they diminish and rarely exceed 2,000 per ml. (Stockmayer 1936). The udder may remain infected for years. About 30 per cent. of samples of raw mixed milk offered for human consumption appear to contain this organism.

Streptococci.—Mastitis streptococci are of various types, the most important being *Str. agalactiae* (see Chapter 24). They are excreted in variable numbers in the milk of cows suffering from mastitis. This disease is extremely common, and affects not only the poorer herds, but even herds producing high-grade milk. Pullinger (1935), for example, found that the milk from 10 out of 12 herds producing Certified milk and 12 out of 14 herds producing Grade A tuberculin-tested milk contained mastitis streptococci when examined at intervals over a period of 15 months. Most streptococci causing mastitis are probably non-pathogenic for man, but occasionally *Str. pyogenes* of human origin invades the udder, and is responsible for an outbreak of scarlet fever or septic sore throat in persons consuming the milk (see Bendixen and Minett 1938, Dublin *et al.* 1943). The milk may also be contaminated directly with these organisms from persons who are either suffering from streptococcal throat lesions, or who are carrying these organisms in their throat or nasopharynx (see Henningsen and Ernst 1939). Most outbreaks of milk-borne streptococcal infection appear to result from the former method of infection. Though pathogenic streptococci must often gain access to milk from human cases and carriers, their rate of multiplication in raw milk at ordinary temperatures is usually too slow for them to render the milk sufficiently infective to cause disease (see Pullinger and Kemp 1937).

Staph. aureus.—This organism is a not infrequent cause of mastitis. It is often found in the apparently healthy udder, but in cows suffering from mastitis it may be present in large numbers in the milk. Its public health importance lies in the fact that, under favourable conditions, it may multiply in the milk and give rise to a toxin capable of producing gastro-enteritis in human beings (see p. 1808).

C. diphtheriae.—This organism occasionally finds its way into the milk from the throat or nasopharynx of a human carrier or case of diphtheria. Very occasionally it becomes implanted on ulcers on the cow's teats. Such an occurrence is peculiarly dangerous, since the milk is uniformly infected (for references, see Goldie and Maddock 1943).

pressure is high, 5000 to 6000 atmospheres, and the exposure prolonged. Under such circumstances the organisms may be reduced in numbers. Spores are more resistant; the spores of *B. subtilis*, for example, survive 20,000 atmospheres. On the other hand, animal cells are killed by pressures of 1800 atmospheres. Increased pressures of carbon dioxide show more bactericidal activity, non-spore-forming bacteria being killed after ninety minutes' exposure of 50 atmospheres. Such a germicidal effect may possibly be a result of increased acidity. The sudden release of high carbon dioxide pressures may result in the disruption of the cells of some bacteria; the gram-negative forms are more readily broken up in this manner than are the gram-positive. Sudden release of pressures of other gases does not have this effect.

CHEMICAL AGENTS¹⁷

The general subject of the effect of chemical agents on bacteria is a broad one and might reasonably be considered as including not only the germicidal chemicals but also the foodstuffs used by these organisms. It is more convenient, however, to consider the latter in terms of the effects of bacteria on their environment in Chapter 4 under the general head of bacterial physiology.

A certain terminology has grown up about this subject matter which requires definition. The synonymous terms *bactericidal* and *germicidal*, which have already been used, are adjectives indicating a bacteria-killing power. The term *bacteriostasis*, or the adjective *bacteriostatic*, denotes a somewhat less drastic effect. A bacteriostatic substance is one which does not kill bacteria but acts in a preservative manner by preventing their growth. An *antiseptic* is a substance which has a preservative action, possibly killing a few of the bacteria exposed to it, but in general acting predominantly in inhibitory fashion. The term *disinfectant* is applied to substances having bactericidal activity and denotes something more vigorous than does antiseptic. These terms are obviously relative, for a substance that is bactericidal in a given concentration may be only inhibitory or antiseptic in lower concentrations. Furthermore, the specificity of a certain compound for some kinds of bacteria may result in its having disinfectant action with regard to one species but only antiseptic action on another.

Water. In general, bacterial cells suspended in distilled water do not survive more than a few hours, although spores will survive for many weeks. Death of the organisms results from a variety of factors one or more of which has been operative in most of the reported experiments. Water from a metal still, for example, often contains sufficient traces of metals to be toxic, and water sterilized in soda or "soft" glass contains alkali dissolved from the glass. Water freshly distilled from hard glass is neutral but upon standing absorbs carbon dioxide from the air and becomes acid. The pH markedly influences the survival of bacteria in water, the death rate increasing on either side of pH 6.0, which appears to be optimum for longevity. Other factors, such as the number of bacteria suspended in a given amount of water, dissolved oxygen and accessibility to oxygen, etc., are likewise known to affect the survival of these organisms. The osmotic pressure of distilled water, which

¹⁷ See Wyss Ann. Rev. Microbiol., 1948, 2, 413.

and bacteria may gain access to the milk in considerable quantities without endangering the health of those consuming it. On the other hand, if pathogenic bacteria find their way into the milk from one of the sources quoted above, no matter in how cleanly a manner the milk is produced, it is a potentially dangerous and unsafe milk.

These conclusions are borne out by epidemiological experience, which has shown that several outbreaks of disease have followed the consumption of milk of the highest standard of cleanliness. Without discussing this subject further, we shall probably be wise to regard cleanliness and safety as two entirely separate attributes of milk.

For human consumption it is desirable, of course, that milk should be both clean and safe. Clean milk is more aesthetically desirable, it has a better flavour, and it keeps longer. Moreover, it is not likely to contain any of those toxic substances resulting from undue bacterial proliferation, which have an irritating effect on the gastro-intestinal tract—particularly of infants (see Park and Holt 1903 and Chapter 72). The fewer organisms there are in milk, and the more bacterial proliferation is checked, the less liable is the milk to give rise to digestive disturbance of this type.

From this it will be clear that *no raw milk can ever be regarded as completely safe for human consumption*. The frequency of disease in cattle, the risk of contamination from human and other sources, and the suitability of the milk itself as a medium for bacterial multiplication, combine to render the consumption of raw milk potentially dangerous. The only satisfactory way of eliminating this danger is by pasteurization or some other form of heat treatment.

The Antibacterial Property of Milk.—Numerous observers have shown that the growth rate of organisms is much slower in clean than in dirty milk. In clean milk there is a lag phase of variable duration before multiplication begins, whereas in dirty milk kept at a suitable temperature the organisms start multiplying almost at once. Jones and Simms (1930) brought evidence to show that clean milk contains an inhibitory substance to which they gave the name of *lactenin*.

Lactenin is present in the whey fraction of the milk. It will not pass through a dialysing membrane. It is inactivated by heating to 80° C., but not by pasteurization. It will remain stable for some weeks at 6° C., and is not digested by trypsin. It is irreversibly inactivated by the exclusion of atmospheric oxygen and by sulphur-containing reducing agents such as cysteine, glutathione, and thioglycollic acid. It is particularly active towards Group A streptococci, which are not only inhibited but actually killed by it. The susceptibility of these organisms to lactenin may explain the infrequency of streptococcal milk-borne outbreaks (see Wilson and Rosenblum 1952); though as most of the outbreaks that do occur are attributable to milk coming from an infected udder, and as lactenin is inactive *in vivo* owing to the low Eh of the tissues, this explanation cannot be more than partially true. Electrophoretic experiments show that lactenin contains two fractions, 1 and 2; these can be concentrated and separated from each other (Auclair and Berridge 1953, Auclair 1954).

Bacteriological Grading of Milk

In public health practice it is usual to grade milk bacteriologically partly according to cleanliness and partly according to safety. We may consider briefly the various methods available for these purposes.

leucocytes, or to some other cause. A mere count of the cells present affords little information about the quality of the milk, and it would be unwise to condemn a milk solely on account of an increased cell content. If long-chained streptococci are found in addition, the probability is that the cow is suffering from mastitis. Much more information can be obtained by a differential count, but the real value of this method lies in the help it gives to the veterinarian who is engaged in a study of the individual cow.

(3) **The Breed Smear Method.**—The general technique of this method is to spread 0.01 ml. of the milk over 1 sq. cm. on a glass slide, to fix and de-fat, to stain with methylene blue, to count the number of individual organisms—the Breed “individual” count—or preferably the number of groups of organisms, individual organisms each being regarded as a group—the Breed “clump” count—in a given number of fields, and finally to calculate the number of organisms in 1 ml. of the original milk. The result obtained affords an index of the total *stainable* organisms present, not necessarily of the total organisms. The chief value of this method lies in its rapidity, which enables an opinion to be formed on the bacterial cleanliness of a milk within a few minutes. It is particularly suitable for use in collecting stations to which milk from individual farms is brought before being mixed and sent up to towns in large rail or road tanks. Unsatisfactory milks, which would taint the rest, can be picked out quickly, and returned to the producer. With a little experience, a mere glance over a few fields is sufficient to indicate the general quality of the milk. The Breed Smear method is also of value to the farm inspector in indicating the probable nature of the defect in unsatisfactory milk. Thus the presence of large masses and clumps of bacteria suggests unsterilized milk utensils; the presence of numerous organisms arranged in pairs or small units suggests inadequate cooling; the presence of long-chained streptococci associated with a high leucocyte count indicates mastitis, and so on (see Breed 1929).

(4) **The Keeping Quality Test.**—The milk is kept in a special bottle at a given temperature, such as 60° F. or 70° F., and examined every 8 or 12 hours until it becomes sour or putrid. The fact that the end-point is largely subjective necessarily exposes the result to a big experimental error. Some degree of objectivity can be introduced by means of the *alcohol-precipitation* or the *clot-on-boiling test*.

If 1 ml. of a 68 per cent. solution of ethanol is added to 1 ml. of the milk and the tube is inverted, precipitation of the casein occurs in milks that are near the souring point. The clot-on-boiling test is carried out by heating 2 ml. of milk in a clean test-tube in boiling water for 5 minutes, samples showing no sign of precipitation are regarded as passing the test.

Though the alcohol-precipitation method is slightly more delicate, both these tests give similar results and are in general agreement with the grading of the samples by taste (see Anderson and Wilson 1945, Rowlands *et al.* 1950). The subjective keeping quality test, though useful for special purposes, is manifestly unsuitable for routine grading, but the clot-on-boiling test has a good deal to recommend it (see Rowlands and Hosking 1951). It is really an indirect test for the measurement of acidity, though a small proportion of milks may clot on boiling in the absence of acidity if enzymes of the rennet type are formed in sufficient concentration—“sweet curdling.” The modified methylene blue reduction test carried out at 60° F. is sometimes used as an indirect measure of keeping quality (see Provan, Dudley and Thomas 1936).

son of the bactericidal power of the various metallic salts on the basis of percentage solution is misleading; equimolecular solutions must be used and the ionization constants taken into consideration. The bactericidal activity of the heavy metal salts is a result of the affinity of the cations for protein material; when the constituent protein of a bacterial cell is precipitated as an insoluble proteinate, the cell dies. Other factors appear to be involved also, however. Guest and Salle¹⁸ have observed that inorganic metallic salts which are only slightly bactericidal individually may become markedly active when mixed to produce an oxidation-reduction system.

The *oligodynamic action of metals* is possibly a result of the solution of the metal to form salts. The destruction of bacteria in contact with or in proximity to a piece of metal is the basis of some methods of disinfection of water. Water may be sterilized by allowing it to seep through a layer of silver-coated sand, and water containing colloidal silver in amounts sufficiently small to defy chemical detection, by calculation about 40 gamma per milliliter, is markedly bactericidal. Such colloidal solutions are prepared by sputtering silver electrodes in water—the so-called catadyn process.¹⁹

The other cations are less toxic than mercury and silver, but even sodium and potassium are toxic to bacteria in sufficiently high concentrations (ca. 2 molar). It is of some interest that the arrangement of cations in an order of their toxicity—mercury and silver at one end and sodium and potassium at the other, with others falling in order in between—corresponds closely to the Hofmeister series and the lyotropic series of Freundlich, in which cations are arranged in the order of their effects on physical properties of proteins, such as coagulation, solubility, viscosity, etc. The toxicity of cations as manifested in solutions containing a single salt may often be neutralized by the presence, in the proper proportion, of another cation. This phenomenon, known as the *antagonistic effect of salts*, has led to the concept and preparation of so-called balanced solutions such as Ringer's solution, Locke's solution and others. Salts not only modify or enhance the toxic qualities of other salts but also exert similar effects on disinfectant compounds of widely different constitution. Sodium chloride, for example, markedly enhances the germicidal qualities of phenol for anthrax spores when present in sufficiently high concentrations.

The part which anions play in the growth and destruction of bacteria is less well known. Some, particularly those containing sulfur, carbon, nitrogen or oxygen, may serve as sources of these elements and of energy, but in appropriate concentrations many are toxic for bacteria.

Oxidizing Agents. Other salts, such as potassium permanganate and the sodium and calcium salts of hypochlorous acid (HOCl), show marked bactericidal activity owing to their properties as oxidizing agents. Mol for mol, hypochlorous acid is one of the most powerful germicides known, and its calcium salt (commonly known as bleaching powder) has a wide use in the treatment of private and small municipal water supplies. Hypochlorous acid reacts with organic compounds containing an amide group with the formation of compounds known as chloramines. These compounds show strong disinfectant properties which are apparently associated with the presence of the

¹⁸ Guest and Salle: Proc. Soc. Exp. Biol. Med., 1942, 51:272.

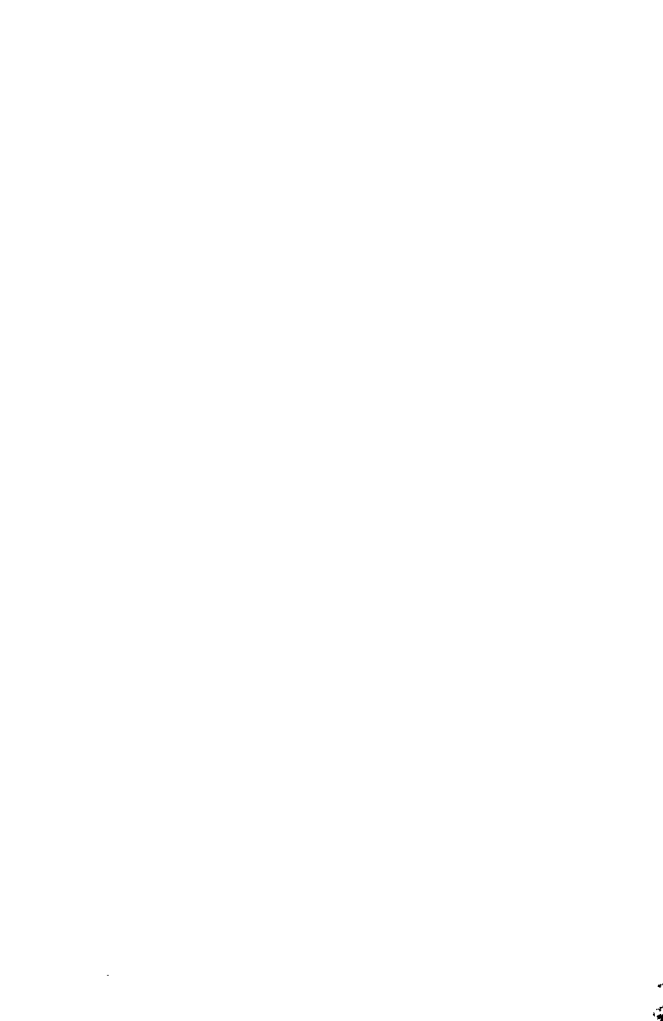
¹⁹ For a discussion see Hoffmann: Arch. f. Hyg. u. Bakt., 1938, 120:147.

(Bardsley 1934); (2) organisms of the *Bact. coli* type in water in this country are very frequently derived from human excretal material, and since human faeces and urine may contain pathogenic bacteria, the presence of any excretal pollution, as indicated by the coliform test, must be regarded as potentially dangerous; and (3) as a rule, coliform organisms do not seem to multiply in water under natural conditions; on the contrary, they tend to die out rather rapidly (Houston 1913). In this country, therefore, the coliform count in water indicates the minimal amount of excretal pollution that has occurred.

When we turn to *milk* we find that (1) a considerable proportion—something like 50–70 per cent.—of raw milks in this country in which coliform bacilli are found contain not the true *Bact. coli* but organisms of the intermediate or aerogenes-cloacæ types. These organisms come mainly, not from faeces and urine, but from soil and grain, and their presence therefore in milk cannot be regarded as an index of excretal pollution; (2) the true *Bact. coli* organisms that are found in milk appear to come either directly from cow-dung and manure, or indirectly from unsterilized milk utensils in which bacterial multiplication has occurred. If they are derived from the latter source, they clearly afford no index of *direct* excretal pollution. If they are derived from the first source, their presence may be considered objectionable on the ground that organisms pathogenic for man are sometimes present in the intestinal canal of the cow. The presence, however, in milk of excretal material of bovine origin must be regarded as very much less dangerous for man than that of human origin. (3) Unlike water, milk affords an admirable medium for the growth of coliform bacilli. If it is kept at a temperature of 50° F. or over, a great increase in their numbers may take place, a rise of several thousand-fold often occurring at 60° F. within 24 hours (see Ayers and Clemmer 1918, Finkelstein 1919, Sherman and Wing 1933). Unless, therefore, the milk has been kept at 40° F. or less, an estimation of the coliform bacilli affords only a very imperfect, and often entirely misleading, index of the extent of the original contamination. With a milk produced and kept under ordinary conditions, it is impossible to tell how many of the coliform bacilli gained access at the time of production, and how many are due to the multiplication of those originally present.

It will thus be seen that none of the three premises on which the scientific application of the coliform test to water in this country is based holds true for milk. Even a differentiation of the coliform group into its constituent types is not likely to help us, since (a) it is impossible to tell how many of the different types were original contaminants and how many have resulted from growth, and (b) organisms of the faecal coli group are not necessarily derived directly from excretal material, but may come from unsterilized milk utensils on which they have been multiplying perhaps for several generations.

The presence of coliform bacilli in small numbers in water or milk is not in itself objectionable; it is merely as an index of the possible accompanying presence of pathogenic organisms that it is of importance. In milk, the coliform test cannot be used as an index of direct excretal pollution, for though it is true that if manure gains access to milk, coliform organisms will probably be found, the reverse conclusion does not hold true. There are so many other sources on the farm for these organisms that their presence in milk cannot be held to justify the conclusion that the milk has necessarily been contaminated with excretal material. The coliform test on milk fails to provide us with that specific qualitative information which it supplies in the case of water, and as a general method of grading, therefore, it seems



peculiarly fitted to gauge the keeping quality of the milk. The plate count yields a *static* picture of the bacterial population: the methylene blue reduction test affords a *dynamic* picture. (For comparison between the two tests see Wilson *et al.* 1935, Nichols and Edwards 1936, Asdrubali 1939, Barkworth, Irwin and Mattick 1941, Pullinger 1945, 1946, Provan and Rowlands 1949, Galesloot 1949, Rowlands *et al.* 1950, Johns 1952.)

(8) **The Resazurin Test.**—The principle of this test, which owes its practical introduction mainly to the work of Ramsdell, Johnson and Evans (1935), is the same as that of the methylene blue test. The test consists essentially in adding to the milk a solution of resazurin to give a final concentration of 1/200,000, incubating at a given temperature, and observing the time taken to bring about a change in the colour of the dye, or alternatively noting the colour reached after a given time. The colour passes from blue, through various shades of purple and mauve, to pink, and ultimately to complete decolorization. The change from blue to pink is due to the irreversible reduction of resazurin to resorufin: the change from pink to colourless is due to the reversible reduction of resorufin to hydroresorufin. The colour can be assessed by a suitable comparator. Numerous methods of performing this test have been described (see Johns and Howson 1940, Davis, Newland and Knuckey 1943). In this country a 10-minute test is used for the control of "platform" milk, that is producers' milk arriving at a creamery, in order to see whether it is clean enough to be bulked with other milk. A longer test is employed by agricultural bacteriologists for the control of the general quality of milk at the time of production.

The advantages of the resazurin over the methylene blue test are mainly two. Firstly, resazurin changes in colour rather more rapidly, and though complete reduction takes as long as or even longer than methylene blue, useful information can be obtained by noting a change in colour from blue to lilac or pink. Secondly, resazurin is more sensitive to the cell content of milk, and is therefore regarded by some workers as of value in the detection of mastitis. The practical importance of these two advantages is dubious. Admittedly a 10-minute test is useful as a screen for grossly contaminated milk; but if the resazurin test is to be used as an index of keeping quality and the reduction of the dye observed to the pink stage, then there is practically no saving of time over the methylene blue test (Anderson and Wilson 1945). Watts and Stirling (1944), in a very careful investigation, found that the resazurin test, even when carried out on individual quarter samples, was of limited value for the routine diagnosis of mastitis, and practically valueless for the detection of milk from infected cows when mixed with normal milk; in fact, as much as 73 per cent. of the herd milk could be derived from cows suffering from mastitis without significantly altering the result of the resazurin test.

The disadvantages of the resazurin test are that the dye is not easy to standardize and is very sensitive to sunlight, that the colour change has to be followed by a comparator, and that the lilac and pink stages are not easily appreciated by persons whose vision is insensitive to red colours. In general terms it may be said that the resazurin test is more influenced by abnormal milk such as colostrum, late-lactation milk, milk from cows with mastitis, and by milk containing organisms with low reducing powers, but that the methylene blue test affords the better index of bacterial contamination of the milk.

(9) **The Laboratory Pasteurization Test.**—This consists in making a plate count

Soaps, the sodium and potassium salts of higher fatty acids, are mildly germicidal in themselves, but probably what disinfectant action they possess may be accounted for on the basis of mechanical removal of microorganisms by emulsification of the lipoidal secretions of the skin in which many bacteria become embedded. The surgical soaps, containing a disinfectant, are not as effective germicides as might be supposed, although somewhat more bactericidal than ordinary soaps.²¹ The slight bactericidal action of fatty acids is apparently attributable to the hydrogen ion. During recent years a group of substances similar to soaps, the sodium alkyl sulfates (such as Drene and others), have come into widespread use. These, like soaps, are anionic detergents. Some of these have been found to inhibit the growth of some bacteria in relatively high concentrations (0.1 per cent), but the activity is markedly selective, gram-positive bacteria being inhibited but gram-negative bacteria not affected.

Reduced surface tension in itself has little detrimental effect on bacteria, although it might be supposed that a germicidal substance which markedly reduced the surface tension of water would be more effective as a result of concentration at the bacterium-water interface. In the case of surface-active germicides it has been observed that wetting action and bactericidal proper-



ties are closely correlated. Whatever the mechanism, it has been found that wetting agents apparently increase the bactericidal action of undissociated phenols.²² In contrast with the soaps and alkyl sulfates, the salts of quaternary ammonium bases are cationic detergents. These have been of interest in recent years and appear promising as germicides, especially those having 12 to 16 carbon alkyl chains. Very many, perhaps a thousand, of these compounds have been synthesized and are marketed under various trade names such as Phemeral, Zephiran, Roccal and the like. These are definite mixtures of related compounds that differ in the number of carbon atoms in the alkyl chain. They are usually most effective on non-spore-forming gram-positive bacteria such as staphylococci and streptococci, and less so on gram-negative forms such as the enteric bacilli. The high degree of bactericidal activity which they show in the phenol coefficient type of test is, however, largely spurious.²³

Ethyl alcohol and ethyl ether, often used as skin disinfectants, are not good germicides. Their effectiveness probably lies in the solution of the lipoidal secretions of the skin and consequent mechanical removal of microorganisms. Absolute alcohol has little or no germicidal activity. The bactericidal activity of alcohol-water solutions increases with the addition of water but 50 per cent alcohol and less has little activity; 70 per cent is the concentration usually used for skin disinfection. Absolute propyl and isopropyl

²¹ See Morton: Jour. Amer. Med. Assn., 1944, 124:1195

²² See the review of surface-active agents by Glassman Bact. Rev., 1948, 12:105

²³ For a discussion of these compounds see Rahn and van Eseltine Ann. Rev. Microbiol., 1947, 1:173.

title of "aggressin," did not long remain unchallenged. Wassermann and Citron (1907) contended that the aggressins were simply products of bacterial growth, or of bacterial autolysis, which could be prepared in the test-tube by suitable methods. Citron (1906) showed that the addition of bacterial extracts to living bacteria increased their virulence; that by the use of such extracts it was possible to immunize animals against experimental infection; and that the serum of such immunized animals conferred passive immunity on others. Bail and Weil (1906) maintained that the immunity produced by Citron with his bacterial extracts was far less effective than that produced by natural aggressins, and that it was different in kind; though it is not entirely clear on what differential criteria they relied in distinguishing anti-aggressin immunity from the varieties previously recognized.

Doerr (1906) criticized the aggressin theory on somewhat similar grounds, but emphasized particularly the fact that large doses of dead bacilli may cause death in experimental animals with the lesions described by Bail as characteristic of the aggressin effect. He also noted that when sub-lethal doses of killed cultures were added to sub-lethal doses of living bacteria the combined inoculum might cause a fatal infection of the aggressive-type. This additive effect did not appear to be in any way specific, but some killed cultures were more effective than others. For instance, dead typhoid or dysentery bacilli were more aggressive than dead staphylococci. In addition, Doerr demonstrated that aggressive exudates did, in fact, contain ordinary bacterial antigens, because a typhoid exudate gave well-marked precipitation with an ordinary antityphoid serum.

Sauerbeck (1907) also criticized Bail's hypothesis. He concluded, from the results of a large series of experiments, that aggressive exudates are not lacking in toxicity, since two or three times the amount that produces the characteristic aggressive effect will kill an animal within a few days in the absence of living bacteria; that the activity of a given aggressive exudate is in no way proportional to the virulence of the organism that produced it, but rather to its toxigenicity; that characteristic aggressive effects can be produced with toxic bacterial products that have been prepared in the test-tube; and that the aggressive action, whatever its cause, is entirely non-specific. His findings as regards the heat-lability of the aggressive bacterial products also differ from Bail's, since he states that they were not reduced in potency till they were heated at temperatures between 55° C. and 70° C.

Dudgeon's (1912) studies of the properties of exudates obtained from natural and experimental infections made it clear that one of the mechanisms in the total aggressin effect was a specific anti-opsonic action.

He obtained exudates from natural infections in man or from experimental infections in animals. The exudate, after the separation of as much material as possible by centrifugation, was mixed with an equal volume of normal serum, or of serum from an immunized man or animal. After 1 hour's incubation at 37° C. leucocytes and bacterial suspension were added to each mixture, the mixtures were re-incubated for 15 minutes and films were prepared from the contents, the degree of phagocytosis being determined in the usual way. In control tubes the exudate was replaced by saline. In each case the opsonic effect of the serum, and of the serum-exudate mixture, was determined against the bacterium causing the infection and against some related organism. The results shown in Table 73 illustrate an effect commonly observed. The exudate came from a subcutaneous staphylococcal abscess; the antiserum was from the infected patient; and the normal serum from an uninfected control. The sera and the serum-exudate mixtures were tested against the strain of staphylococcus grown from the abscess and against a strain of *Bact. coli*.

The exudate alone had a slight opsonic effect, but it specifically diminished the opsonic action of normal or of immune serum on *Staph. aureus*, leaving unaffected their opsonic action on *Bact. coli*. The substances responsible for this specific anti-opsonic effect were

Dyes

alcohols are likewise ineffective but show activity in aqueous solution, while absolute methyl alcohol is said to be bactericidal.

Dyes. The dyes are widely used in bacteriology both for staining purposes and as indicators. In addition, many of them show a marked bacteriostatic and bactericidal activity which is often specific in that it is manifested against one organism and not another. The incorporation of an appropriate dye in a medium will render it selective, i.e., it will favor the growth of some species of bacteria and inhibit that of others. In general, this specificity is correlated with the gram reaction, the gram-negative organisms are, for the most part, much less sensitive to dyes than are the gram-positive species. The activity of these compounds is affected by pH, the toxicity of the acid dyes increasing with acidity and that of the basic dyes increasing with alkalinity.

Many of the dyes such as the thiazins, oxazins and azo dyes are not particularly toxic for bacteria, dilutions of 1:1000 or less being required to inhibit growth. A number of the triphenyl methane dyes are, on the other hand, inhibitory in high dilutions. Malachite green, for example, inhibits the growth of *B. subtilis* in dilutions of 1:4,000,000 and staphylococci in 1:1,000,000, while higher concentrations, i.e., 1:30,000 to 1:40,000, are required to inhibit the colon and typhoid bacilli. Victoria green, a dichlor derivative of malachite green, is bacteriostatic to about the same degree. Brilliant green is active in even higher dilutions, inhibiting *B. subtilis* in a dilution of 1:15,000,000 and staphylococci in 1:4,000,000 and the typhoid and colon bacilli in 1:500,000.

The bacteriostatic properties of the triamino triphenyl methane dyes, the so-called rosanilins, are apparently associated with the substitution of alkyl groups in the amido side chains. Basic fuchsin, a mixture of the unsubstituted simple dyes rosanilin and pararosanilin, is relatively weakly bacteriostatic, dilutions of 1:500,000 being required to inhibit the growth of *B. subtilis*. Acid fuchsin, a mixture of various sulfonated derivatives of basic fuchsin, is likewise only weakly inhibitory and was formerly widely used in media as an acid indicator under the name of Andrade's indicator. On the other hand, methyl violet,²¹ a mixture of tetra-, penta- and hexamethyl pararosanilin, is markedly bacteriostatic and completely inhibits the growth of bacteria such as staphylococci, diphtheria bacilli and others in dilutions of 1:1,000,000 to 1:5,000,000. Approximately 150 times as much dye is necessary to suppress growth of the less sensitive gram-negative bacteria such as the colon and typhoid bacilli. There appears to be a correlation of bactericidal activity and basicity in the dyes as chemotherapeutic agents in recent years.²²

The acridine dyes, acriflavine and trypanflavine, have been of particular interest because of their therapeutic significance. The former is actively bacteriostatic in dilutions as high as 1:3,000,000 and the latter inhibitive to

²¹ Gentian violet is a more or less impure mixture of methyl violet and deinox. Crystal violet hexamethyl pararosanilin, is one of the constituents of methyl violet.
²² Cf. Mellmann *Biochem. Jour.*, 1941, 35-1311. Rubbo, Albert and Maxwell: *Brit. Jour. Exp. Path.*, 1942, 23-69. Russell and Falconer: *Lancet*, 1943, ii 580. Browning: *Brit. Med. Jour.*, 1943, i 263. Albert, Rubbo, Goldacre, Daves and Starr: *Brit. Jour. Exp. Path.*, 1945, 26-160.

TABLE 194

Designation	General Conditions	Bacteriological Requirements	Remarks
Tuberculin tested	Must come from an Attested herd, or from a herd in which veterinary inspection and tuberculin tests are carried out as specified by the Minister of Agriculture and Fisheries.	Must not decolorize methylene blue in $4\frac{1}{2}$ hr. 1 May to 31 October, or in $5\frac{1}{2}$ hr. 1 Nov. to 30 April. ¹	(i) If bottled on farm, may be labelled Tuberculin Tested (Farm Bottled). (ii) If pasteurized, must not decolorize methylene blue in 30 min., ² or give a phosphatase reading of over 2.3 Lovibond units (iii) If sterilized, must pass the turbidity test.
Accredited	Must be derived from a single herd only. Herd must be subject to veterinary inspection and must not contain any animal known to react to tuberculin.	Same as for Tuberculin Tested milk. ¹	Designation valid only till 1 October 1954 when this grade was abolished.
Pasteurized	Milk must be kept at a temperature (i) of 145-150° F for at least 30 min., or (ii) of 161° F. for at least 15 sec., and be immediately cooled to 50° F. or below.	Must not decolorize methylene blue in 30 min., ² or give a phosphatase reading of over 2.3 Lovibond units.	Designation must be on cords must be preserved for at least a month. H.T.S.T. plant must be provided with an automatic flow-diversion valve. Sale of loose milk forbidden after 1 October 1954
Sterilized	Milk must be kept at a temperature of not less than 212° F for a time sufficient to ensure compliance with the turbidity test.	Must pass the turbidity test.	Sterilizing plant must be approved by the licensing authority, and must be provided with the specified thermometers and pressure gauges. Milk must be heated in bottles, and the bottles must be sealed afterwards with an air tight seal.

¹ If the sample is taken before the milk has been placed in bottles or other containers for delivery to the consumer (i.e. *producers' milk*), it must be transferred to the laboratory in an

milk, and a sample of as an evening milk be cooled to between before examination

The bottle or container for delivery to the consumer (i.e. *consumers' milk*) must be transferred to the laboratory in an insulated container. If it cannot be delivered to the laboratory within this time, it must be packed in ice. If the sample cannot be examined immediately it arrives at the laboratory, it must be cooled to between 32° and 40° F. and kept at this temperature for not more than 24 hours before examination. Any sample not reaching the laboratory on the day on which it is taken must be discarded unless it has been kept packed in ice since the previous day.

² The sample must be pac

staphylococci and similar organisms in 1:2,000,000 and to the relatively fragile gonococcus in dilutions of 1:10,000,000 to 1:50,000,000. The gonococcus is killed by exposure to the dye in dilutions of 1:80,000 to 1:400,000 within two or three minutes.

Gaseous Disinfectants. The use of bactericidal gases for the disinfection of rooms, dwellings and the like (fumigation or terminal disinfection) has declined markedly in recent years with no coincident increase in the prevalence of infectious disease. The commonly used gases, formaldehyde and sulfur dioxide (generated by burning flowers of sulfur), are probably not bactericidal as gases but in aqueous solution and are effective, therefore, only in the presence of adequate amounts of moisture (a relative humidity of 60 per cent or higher). Sulfur dioxide in aqueous solution (sulfurous acid) probably owes its germicidal qualities to its acidic nature. Formaldehyde, usually sold under the trade name of formalin (a 33-40 per cent solution of the gas in water), has greater penetrating power and is a more effective germicide than sulfur dioxide. Other gases such as hydrogen cyanide have little or no effect on bacteria. Although the value of terminal disinfection is open to serious question, that of disinfestation is well established and the gases, hydrocyanic acid in particular, are widely used for the destruction of rats aboard ship, etc.

Aerosols. The use of aerosols, bactericidal compounds finely dispersed in the air, for the destruction of air-borne pathogenic bacteria has been developed in recent years, especially by Robertson and his co-workers.²⁶ These investigators first used propylene glycol as a vehicle for bactericidal compounds but control experiments indicated that the glycol alone (volatilized by heat) was equally effective. Triethylene glycol is the glycol now most commonly used. The glycol is relatively non-toxic and the vapor may be breathed with impunity, but bactericidal concentrations of 50 per cent or higher are attained when droplets come in contact with suspended microorganisms. The rate of destruction of air-borne bacteria is in agreement with the assumption that the glycol molecules in the vapor state condense on the bacteria-containing droplets, and efficacy is associated with a low vapor pressure, high hygroscopicity and, of course, toxicity for the bacterial cell substance.²⁷ Other workers have used dispersed hypochlorite and ultraviolet irradiation to kill air-borne bacteria but these methods are not too promising. These results are of considerably more than ordinary significance in that they make possible the control of air-borne infection (p 231); the respiratory diseases have hitherto spread in the human population without hindrance.²⁸

The Specificity of Disinfectants. The marked specificity of the bacteriostatic and bactericidal activity of the dyes has been referred to above. The property of differential toxicity is not confined to these compounds alone, however, but is exhibited to some degree by many of the bactericidal chemicals. The hypochlorites, for example, while powerful germicides for most bacteria, have little effect on the tubercle bacillus. In general, the salts of the heavy metals are least specific in their action and dyes the most, with other

²⁶ Robertson *et al.*: *Jour. Exp. Med.*, 1942, 75:593; *ibid.*, 1943, 78:387; *Science*, 1943, 97:142.

²⁷ Puck: *Jour. Exp. Med.*, 1947, 85:729, 741.

²⁸ See, for instance, the review in *Bull. U. S. Army Med. Dept.*, 1946, 5 538.

by Pasteurized milk, the milk had been filled into contaminated churns or bottles; in 1 the milk before pasteurization was heavily contaminated with *Staph. aureus* with the result that the milk after processing probably contained undestroyed staphylococcal enterotoxin; and in 1 outbreak caused by Pasteurized milk and

TABLE 195

RECORDED OUTBREAKS OF MILK-BORNE DISEASE IN GREAT BRITAIN IN 1938-52
(EXCLUDING TUBERCULOSIS, UNDULANT FEVER AND Q FEVER, AND OUTBREAKS
DUE TO MILK PRODUCTS SUCH AS ICE-CREAM, CHEESE, AND CREAM BUNS)

Disease	No of Outbreaks	No of Outbreaks in which Number of Persons affected is stated	No of Persons affected in these Outbreaks
Scarlet fever and septic sore throat	14	14	815
Diphtheria	6	6	37
Typhoid fever	8	8	57
Paratyphoid fever	13	13	309
Dysentery	21	20	2,630
Gastro-enteritis	48	44	3,109
Infectious hepatitis	2	2	153
Total	112	107	7,110

in the 2 outbreaks caused by Sterilized milk the milk was heavily contaminated with aerobic spore-bearing organisms which had apparently grown in the milk after processing

In the United States Armstrong and Parran (1927) recorded 612 milk-borne outbreaks occurring during the preceding 20 years, and Andrews and Fuchs (1918) recorded the figures for the 23 years 1923-45 (Table 196).

TABLE 196

OUTBREAKS OF MILK-BORNE DISEASE IN THE UNITED STATES OF AMERICA,
1923-45 (modified from Andrews and Fuchs 1918).

Disease	No of Outbreaks	No of Cases	No of Deaths
Scarlet fever and septic sore throat	202	19,190	182
Diphtheria	18	324	13
Typhoid fever	440	7,449	541
Paratyphoid fever	27	1,063	22
Dysentery	20	1,413	20
Gastro-enteritis	196	9,780	19
Miscellaneous	18	683	4
Total	921	39,902	801

This table again affords merely a conservative estimate of the frequency of milk-borne epidemics, and from previous experience there is reason to believe that many outbreaks are not recognized or are not recorded (Brooks 1933). It will be noted that the relative incidence of enteric fever is very much higher than in Great Britain.

Dolman (1941) in Canada collected records of 67 milk-borne outbreaks during

The Chemotherapeutic Drugs

compounds lying between these two extremes. Certain slow oxidizing agents such as potassium dichromate exert a selective bacteriostatic effect on gram-negative bacteria, and iodine is more efficacious against these microorganisms. Similarly, among the long chain aliphatic bases, the less strongly basic amines act on gram-positive bacteria for the most part, whereas the gram-negative organisms are more susceptible to the action of the stronger bases such as guanidines and quaternary amines.²⁹

Although it might be supposed that the specificity of disinfectants is an undesirable quality, it will be apparent on second thought that specific toxicity is often highly desirable. For example, a compound which is equally effective in the disinfection of both bacteria and tissue cells could not be used to advantage.

The Chemotherapeutic Drugs. The phenomenon of specificity also assumes great practical significance as the basis of chemotherapy of infectious disease. Ehrlich's discovery of salvarsan came as a result of a search for a "magic bullet"—a compound strongly germicidal for a given microorganism yet sufficiently non-toxic to the host that it could be injected into the tissues in effective concentrations. The discovery of chemical structures having these properties has been purely a matter of trial and error rather than rational theory, but once discovered, the active portion of the molecule may, of course, be modified to increase efficacy. decrease toxicity, etc. Successful chemotherapy has, in the past, been confined to certain spirochetoses and protozoan infections, the compounds showing activity including the arsenicals, compounds of antimony and of bismuth, certain dyes such as trypan red and the flavines, synthetic compounds such as atabrine and plasmochin, and certain naturally occurring substances such as quinine. Until relatively recently the bacterial diseases appeared to be, for all practical purposes, resistant to chemotherapy.

With the observation that the azo dye prontosil had marked chemotherapeutic activity in streptococcus infections, and the discovery of the active portion of the molecule, *p*-aminobenzene sulfonamide (sulfanilamide), a new series of compounds, known as sulfonamides, was found to be highly efficacious in the treatment of many bacterial infections. Many derivatives of *p*-aminobenzene sulfonamide have been prepared, radicals being attached to the nitrogen of the sulfonamide group, which show different solubilities, degrees of ionization, toxicity, rates of absorption and excretion, associated with differences in chemotherapeutic efficacy.³⁰

These compounds are bacteriostatic rather than bactericidal and appear to function *in vivo* by suppressing bacterial multiplication, the invading microorganisms being destroyed by the body defences, notably through phagocytosis by the cells of the macrophage system (p. 232). They do not affect the bacterial toxins to a significant degree.³¹

²⁹ Fuller, *Biochem. Jour.* 1942, 36 548.
³⁰ Cf. Bell and Robin, *Jour. Amer. Chem. Soc.*, 1942, 64 2905.

³¹ That is to say, there is apparently no clinically important effect, however, cf. Carpenter and Harbour, *Proc. Soc. Exp. Biol. Med.*, 1939, 41, 354, Hutter and Zahl, *Science*, 1942, 96 563.

country two methods are permitted. The Holder process consists in raising the milk to a temperature of 145–150° F., holding it at this temperature for 30 minutes, and immediately cooling it to 50° F. or below. If this process is carried out in properly designed plant free from mechanical defects (see Scott and Wright 1935, Dalrymple-Champneys 1935), and supervised by intelligent and conscientious operatives, it can be relied upon to destroy all pathogenic organisms in the milk. In the so-called High Temperature Short Time process the milk must be kept at a temperature of not less than 161° F. for at least 15 seconds and then cooled to 50° F. or below. (For description of planning and operation of pasteurizing plants see Report 1953.)

The adequacy of heat treatment may be controlled by the *phosphatase test* (Kay and Graham 1935, Kay, Aschaffenburg and Neave 1939, Memo. 1943, Report 1944), which depends on the fact that the enzyme phosphatase, which is normally present in milk, is destroyed by a temperature of 145° F. within 30 minutes or of 162° F. within 15 seconds. The test is carried out by incubating the milk in the presence of a buffered solution of disodium phenyl phosphate. The amount of phenol that is liberated is measured by adding Folin and Ciocalteu's reagent and estimating the depth of the resulting blue colour by a Lovibond tintometer or, more simply and approximately, by a Lovibond comparator. Raw milk gives figures of about 50 blue units, but in properly pasteurized milk the number of units is reduced to 2·3 or less. This is a most valuable test and can be used in practice to ensure that the processing of the milk is carried out satisfactorily. Phosphatase is slightly more resistant to heat than the tubercle bacillus, so that a negative phosphatase result is strong presumptive evidence in favour of the safety of the milk.

The objections that have been raised to pasteurization, though often made on apparently scientific grounds, are determined almost entirely by economic or political motives. The farmer, especially the producer-retailer, may not obtain such a high return for his milk if he has to pasteurize it himself or send it to a pasteurizing depot as if he sells it directly to the customer in its original raw and possibly dangerous state. Producer-retailers, moreover, are afraid that pasteurization will lead to their being gradually absorbed by the big milk combines, which can of course afford to pasteurize and bottle their milk under very much more favourable conditions than is possible for a small producer. Though appreciating the force of these motives, we must not allow the public health of the nation to be sacrificed to the financial interests of a small section of the community.

One of the chief objections that has been raised is that pasteurization lowers the nutritive value of the milk. The evidence for and against this contention has been reviewed by several workers (see Stirling and Blackwood 1933, Report 1934, 1936, 1937–39, Bendixen *et al.* 1937, Wilson 1942). The main effects of pasteurization by the Holder method at 145°–150° F. for half an hour followed by immediate cooling to 50° F. or below are as follows: (1) the cream line is reduced by about 10–30 per cent. (2) About 5 per cent. of the lactalbumin is coagulated. (3) There is a diminution of about 5 per cent. in the soluble calcium and phosphorus. (4) There is some destruction, amounting usually to about 10 per cent., and at the most to 25 per cent., of Vitamin B₁. (5) There is a variable diminution in the Vitamin C content, dependent on the degree of previous exposure of the milk to light, the presence of copper in the pasteurizing plant, and the amount of dissolved oxygen; at present, the average reduction is about 20 per cent. (6) Some of the iodine

stantially correct.³⁴ For example, the bacteriostatic action of a wide variety of derivatives of sulfanilamide is nullified by *p*-aminobenzoic acid and related compounds which will give it on hydrolysis. The relationship between the antagonist and drug is a quantitative one; the ratios usually reported vary from 1:1000 to 1:26,000 but are considerably nearer unity if the presence of antagonist in the test medium and the ionic concentration of the drug are taken into consideration.

It may be noted that, as a corollary, specimens of blood, urine or other body fluids taken from an individual undergoing sulfonamide therapy may contain sufficient amounts of the drug to inhibit bacterial growth when they are cultured. The inhibiting effect may be avoided by including *p*-aminobenzoic acid in the culture medium; a concentration of 5 mg. per 100 ml. is adequate.

With generalization of the idea of competitive inhibition of essential metabolic reactions, antagonisms to other bacterial vitamins may be predicted and tested. This has been done, especially by McIlwain. It has been found that the modification to produce an analogue may vary and may include the replacement of a carboxyl group with a sulfonic acid or sulfonamide radical, or by a ketone, by the substitution of one atom for another in the ring system of aromatic metabolites, the replacement of alkyl side chains of ring systems with halogens, etc. For example, sulfonic acid and sulfonamide analogues of nicotinic acid and pantothenic acid have been prepared by substitution of $-\text{SO}_3\text{H}$ or $-\text{SO}_2\text{NH}_2$ for a carbonyl group and have been found to be specifically antagonistic and inhibitory. Similar results have been obtained with pyriethamine, the pyridine analogue of thiamine. The reverse kind of experiment has been carried out also. Iodinin, the di-N-oxide of a dihydroxy-phenazine, is a pigment produced by *Chromobacterium iodinum* which inhibits the growth of certain bacteria. The inhibitory effect can be nullified by certain anthraquinones and naphthaquinones, from which it may be inferred that quinones play some part in bacterial metabolism, possibly in the transport of hydrogen. As yet none of the analogues tested have had practical chemotherapeutic activity though some effect may be observed; large doses of thiopanic acid (pantyltaurine, an analogue of pantothenic acid) protects rats against lethal doses of hemolytic streptococci.

It may be noted in passing that the generalization of this theory seems to remain valid when carried over to higher organisms. Thus, symptoms of vitamin deficiency may be produced in experimental animals by feeding an analogue of the vitamin, e.g., pyriethamine, glucoascorbic acid, iso-riboflavin and the phenazine analogue of riboflavin, β -acetyl pyridine (an analogue of nicotinic acid).

The mechanism of competitive inhibition is in most cases more complex than here indicated. It is frequently assumed that analogues compete with metabolites acting as coenzymes, but in general the evidence indicates that the competition occurs when the metabolite acts as a substrate rather than a coenzyme. As a substrate, the metabolite is synthesized into a more complex molecule which functions as a coenzyme, or it may be degraded prior to synthesis. Thus, *p*-aminobenzoic acid is a part of folic acid and competition

³⁴ See the reviews by Wooley: *Advances in Enzymology*, 1946, 6:129, *Physiol. Rev.*, 1947, 27:308; Hotchkiss: *Ann. Rev. Microbiol.*, 1948, 2:183

The cleanliness of churns and bottles can be assessed by means of a plate count carried out on suitable rinsings. The method of examining bottles is described in full by Hobbs and Wilson (1913) in their report on the comparative efficacy of different methods of washing and sterilizing bottles. Briefly it consists in taking 6 or 12 representative bottles, adding to each 20 or 30 ml. of $\frac{1}{4}$ strength Ringer's solution according to the size of the bottle, rolling the bottle to ensure that the whole of the internal surface is thoroughly wetted, allowing the bottle to stand for 15-30 minutes, again rolling it, and then preparing duplicate plates each with 5 ml. of the rinsings. The plates are prepared with Yeastrel milk agar (Memo 1937), and are incubated one at 22° C. and the other at 37° C. for 3 days, after which the colonies are counted. The following standards are generally accepted in this country:

Less than 600 colonies per bottle	.	.	.	Satisfactory
600 to 2000	"	"	"	Fairly satisfactory
Over 2000	"	"	"	Unsatisfactory

The examination of churn rinsings is carried out in an essentially similar way.

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The Mechanism of Antibacterial Action

apparently occurs in the synthesis of the vitamin. Similarly, pyriothiamine does not compete with thiamine pyrophosphate when the latter functions as co-carboxylase, but rather competition appears to occur in the synthesis of the coenzyme from thiamine. It was early assumed that, if a compound is required by certain bacteria, it is an essential metabolite for other bacteria also which do not show the requirement because they are able to synthesize it, it would appear to follow that a drug could compete with a vitamin either preformed or after synthesis by the microorganism. It is not quite so simple as this, however, for it has been found that bacteria which do not require thiamine, for example, are able to split pyriothiamine into pyrimidine and pyridine in the same way that thiamine is split into pyrimidine and thiazole components, and that organisms which cannot synthesize thiamine also lack the ability to split pyriothiamine and are susceptible to its inhibitory activity. Such somewhat indirect relationships to the better known metabolic reactions of bacteria probably account, to some extent at least, for evidence which appears to be inconsistent with a hypothesis of direct competitive inhibition.

The Effects of Antibacterial Compounds The ways in which antibacterial substances affect the cell so as to produce either irreversible changes in the cell organization resulting in death, or an inhibition of processes essential to reproduction, vary. Available evidence indicates, as might be expected, that a variety of effects may be produced by oxidizing agents, a saturation of thiol groups of protein by, for example, the substitution of halogens such as iodine in the ring structures of aromatic amino acids, obviously alters the organization of the cell on a macromolecular level to such an extent that normal metabolism cannot continue. Bactericidal substances of the phenol group apparently act in this way in that phenol is surface-active and by orientation of the hydroxy groups reacts with free amino groups of the cell proteins.²¹ Even those substances which appear to be general protoplasmic poisons, however, may show some specificity of action, there is evidence that chlorine, for example, first affects a triosephosphate dehydrogenase system.²²

Or the effect may be somewhat more localized as in the case of the basic dyes which react with nucleotides, it is not unreasonable to suppose that flavine, is antagonized by nucleotides, it is not unreasonable to suppose that the hereditary mechanisms of the bacterial cell are made up of nucleoprotein and their inactivation by nucleotides, it is not unreasonable to suppose that inhibits proliferative processes. Similarly, the surface-active detergents are antagonized by lipids, e.g., the anionic detergents by cephalin, and there is some reason to believe that they alter the permeability of the cell wall to such an extent that the cell dies.

Interference, by competitive inhibition or otherwise, with specific metabolic reactions is, perhaps, more obvious when these are essential to the metabolism of the cell. Such metabolic reactions may be the energy yielding oxidations of the respiratory process and, as indicated elsewhere, the bactericidal efficacy of many compounds may be estimated as accurately by inhibition of oxygen

²¹ See Fox and Lodge *Trans. Faraday Soc.*, 1945, 41:359.

²² Green and Stumpf *Jour. Amer. Water Works Assn.*, 1946, 38:1301.

uptake in respirometers as by methods using death as an end point. Many of the important antibacterial substances, however, do not appreciably affect the respiratory mechanisms and there is reason to believe, in some cases at least, that they interfere with synthetic mechanisms; for example, there is some evidence that the sulfonamides interfere with synthesis. Other compounds, such as azide and dinitrophenol, actually increase oxygen uptake, in the presence of carbohydrate substrates, but the increased oxidation is due to a prevention of assimilation of carbon by the cell. It will be clear that, while information as to the precise point of attack of antibacterial substances on cell metabolism is far from complete, a variety of mechanisms is not only possible but operative.

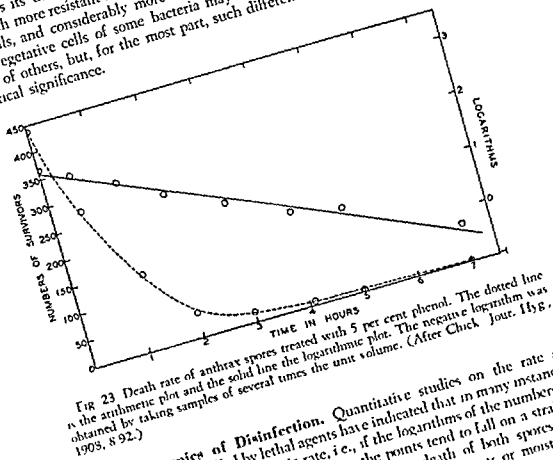
Potentiating and Interference Effects. As yet unexplained are the results of mixing antibacterial substances. In some cases, of course, the antibacterial activity of two substances is simply additive. In others, however, the effect is potentiating in that the activity of the combined substances is greater than a purely additive activity, and the activity of some compounds may be enhanced by mixture with inactive ones. Such potentiating effects are observed in the combination of penicillin (p. 131) with gramicidin (p. 128) or sulfonamides, or in the combination of sulfonamides with azochloramide. The activity of sulfonamides may be enhanced by mixture with inactive substances such as carbamates, urea, asparagin, and the like. In still other instances, notably the incorporation of bactericidal substances with soaps, the combined effect is less than an additive one.

Factors Influencing Disinfection. The process of disinfection or bacterial death is often, in part at least, a chemical reaction and is, therefore, subject to a variety of influences which affect the velocity of such reactions. The most important of these influences is the concentration of the reacting substances, *i.e.*, the concentration of disinfectant and the numbers of bacteria present. The effective concentration of disinfectant is, in turn, dependent upon two other factors, first, the presence of moisture, which makes possible coagulation by heat, and ionization of the bactericidal salts, and acts as a solvent and suspending medium in which there may be intimate contact between the disinfectant and the microorganism; and, second, the presence of extraneous organic matter. Many chemical disinfectants act through a combination with the protein of the cell and, if extraneous organic matter is present, will, of course, react with this inert material, thereby reducing the effective concentration. Disinfectants vary widely in the degree to which their bactericidal properties are affected by organic matter. The salts of the heavy metals are rapidly precipitated by organic material, while compounds such as phenol and the cresols are only slightly affected. The rate of destruction by heat is also affected by the presence of organic matter—organisms embedded in a mass of fecal material, for example, are protected from heat for a short time. The process of disinfection by germicides or by heat is influenced by temperature, the velocity of the reaction increasing with rise in temperature. The pH likewise influences the rate of bacterial destruction not only by heat but by many chemical compounds, the velocity, in general, being least at neutrality and increasing with increase in acidity or alkalinity. A number of other factors such as the presence of salts, etc., affect the rate of disinfection but generally not sufficiently to be of practical importance.

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The Dynamics of Disinfection

From the practical point of view, the time of exposure of bacteria to a given disinfectant is of considerable significance and, of course, bears an inverse relation to the rapidity of killing. The time allowed for the destruction of bacteria is determined not only by the factors discussed above but also by the kind of bacteria that are to be killed. In certain cases the specificity of a disinfectant may be so marked that it must be taken into consideration. For example, the relative toxicity of hypochlorite for the tubercle bacillus referred to above precludes its use in the disinfection of tuberculous sputum. Bacterial spores are much more resistant to heat and chemical disinfectants than are the vegetative cells, and considerably more time must be allowed for their destruction. The vegetative cells of some bacteria may be somewhat more resistant than those of others, but, for the most part, such differences are too small to be of practical significance.



The Dynamics of Disinfection. Quantitative studies on the rate at which bacteria are killed by lethal agents have indicated that in many instances the organisms die at a logarithmic rate, i. e., if the logarithms of the numbers of viable organisms are plotted against time, the points tend to fall on a straight line. This phenomenon has been observed in the death of both spores and vegetative cells under the influence of chemical disinfectants or moist heat and also occurs in the death of bacteria in old cultures. The velocity of the reaction, the slope of such a line, depends upon the concentration and kind of disinfectant, the nature of the organisms—whether spores or vegetative cells—and other factors which influence the process of disinfection. This logarithmic rate likewise describes the course of a monomolecular reaction, and this fact has led some to conclude that bacterial death is a monomolecular chemical reaction. Although the killing of bacteria by some disinfectants is undoubtedly

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a chemical process, as for example the precipitation of constituent protein as proteinates of heavy metals, the evidence does not justify the conclusion that the reaction is monomolecular. The fallacy is the old one of confusion of correlation and causality under the slightly different guise of description and mechanism. Experimental evidence has indicated that while such semilogarithmic plots may often be fitted with a straight line, others are best fitted by sigmoid curves, the death rate being more rapid in the beginning in some cases and more rapid at the end in others or even highly irregular. Some workers³⁷ have concluded that one of the more important determining factors is a graded biological resistance of the cells in the bacterial population, an explanation that is supported by general biological considerations. Undoubtedly the phenomenon of bacterial death is a complex of interrelated factors whose admixture with the mechanics of the mass law results in the parameters determined by mathematical means. Of practical importance, however, is the fact that in disinfection by chemicals and by heat there is a minority of cells, possibly more resistant, that survive long after the majority have perished and that must be destroyed in order to obtain complete sterilization.

The Standardization of Disinfectants. The relative bactericidal efficiency of the chemical disinfectants is a point of considerable practical importance. The value of a quantitative method for the determination of the killing power of germicides was recognized early in the development of bacteriology, and experimental investigation led to the establishing of a standardized technique which made possible the determination of the bactericidal power of a given chemical compound relative to that of phenol. The numerical value so determined is termed the *phenol coefficient* and is presumed to indicate whether, and to approximately what extent, the unknown is a better or poorer germicide than phenol. Later methods of standardization have grown out of this procedure. The phenol coefficient of a disinfectant used against the typhoid bacillus is calculated as follows:

Divide the greatest dilution of the disinfectant capable of killing *S. typhi* in ten minutes but not in five minutes by the phenol dilution which so kills this, and divide these figures one into another. In order not to convey a false idea of the accuracy of the method the coefficient is calculated to the nearest 0.1 point if under 1, to the nearest 0.2 point if between 1 and 5, to the nearest 0.5 point if between 5 and 10, and to the nearest 1.0 point if between 10 and 20.

For example, if a 1:90 dilution of phenol kills in 10 minutes but not in 5, and a 1:100 dilution does not kill in 10 minutes, the former is taken as the end point; and if a 1:350 dilution of the unknown disinfectant kills in 10 minutes but not in 5, while 10 minutes' point is the 1:350 dilution. The phenol 3.89 or 3.9, and the unknown has, by The conditions regarded as standardized, have been defined by the Food and

Drug Administration.³⁸

The effect of extraneous organic matter on the bactericidal power of a disinfectant is commonly taken into consideration by carrying out the test with and without added organic matter. Three per cent of dried fecal matter or dried yeast may be added to the bacterial suspension or the organisms may be

³⁷ Knaysi Jour. Inf. Dis., 1930, 47:293.

³⁸ United States Department of Agriculture Circular No. 198. 1931.

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- amersfoort. *See* *Salmonella*
- amethystinus. *See* *Chromobacterium*
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Note.—For the less common species of *Salmonella*, see pp. 823-848

The Standardization of Disinfectants

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BACTERICIDAL ACTIVITY OF DISINFECTANTS*

STANDARDIZATION OF DISINFECTANTS												
BACTERICIDAL ACTIVITY OF DISINFECTANTS*					Skin Sterilization†							
Compound	Type	Phenol Coefficient‡		Strept viridans		Bact coli		Compound	Number of skin grafts	Per cent sterile		
		Staph aureus		50% serum		50% serum						
		Water	50% serum	Water	50% serum	Water	50% serum					
Merphenyl nitrate (1:1000 aqueous)	R ₁ A	0.409 614	0.133 200	0.077 77	Not done	0.250 375	Not done	0.272 408	0.044 66	Merphenyl nitrate tincture 1:1500	100	0
Merthiolate (1:1000 aqueous)	R A	0.188	0.077	0.31	Not done	0.80 400	Not done	0.043	0.03 30	Merthiolate tincture 1:1000	30	6
Metaphen (1:500 aqueous)	R A	0.375 (87)	0.155 77.5	0.22 111	Not done	0.025 1.25	Not done	0.040 2.0	0.02 20	Metaphen tincture 1:200	50	80
Mercurochrome (1:50 aqueous)	R A	0.040 22	0.022	0.062 62	Not done	0.043	0.02 20	0.150 150	0.020 20	Mercurochrome tincture 1:50	40	10
Hexylresorcinol (1:1000 aqueous)	R A	0.063 63	0.022 22	0.125 125	Not done	0.175 175	Not done	0.355 5	0.35 5	Phenol 2% aqueous	30	0
Iodine tincture (1% in alcohol solution)	R A	20 286	0.355 5	0.112	Not done	10 143	Not done	10 143	0.35 5	Alcohol acetone solution	40	80

* Selected from the data of Lampitt, Campbell and Reames of the University of Chicago.

† Determined on basis of killing in five but not in ten minutes.

‡ The disinfectant is applied to an area of shaved skin, a piece of which is then excised and cultured in parentheses.

§ Relative phenol coefficient determined by dilution of the disinfectant as sup.

|| Absolute phenol coefficient based on the pure compound.

- Bacillus graminis.* See *Actinomyces granulosis.* See *Bacterium grunthal.* See *Bacterium gypsoides.* See *Actinomyces hæmoglobinophilus canis.* See *Hæmophilus canis*
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havana. See *Salmonella*
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helcogenes. See *Vibrio*
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horsham. See *Salmonella*
hvittingfoss. See *Salmonella*
icteroides. See *Salmonella typhi-murium*
immobilis. See *Bacterium indicus.* See *Chromobacterium*
infantis. See *Salmonella*
influenzæ. See *Hæmophilus*
intestinale. See *Thermobacterium*
inverness. See *Salmonella*
java. See *Salmonella paratyphi B*
javana. See *Salmonella*
jena. See *Salmonella enteritidis*
kaukasus. See *Lactobacillus caucasicus*
kentucky. See *Salmonella*
khartoumensis. See *Bacterium*
kiel. See *Salmonella*
kielensis. See *Chromobacterium*
koclin. See *Salmonella*
kunzendorf. See *Salmonella cholerae-suis*
lactis. See *Lactobacillus*
lactis aerogenes. See *Bacterium aerogenes*
lactis erythrogenes. See *Chromobacterium*
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leptisepticus. See *Pasteurella*
lepræ. See *Mycobacterium*
lexington. See *Salmonella*
lignieresii. See *Actinobacillus*
litchfield. See *Salmonella*
lomita. See *Salmonella*
london. See *Salmonella*
luteus. See *Micrococcus*
macallen. See *Salmonella*
madampensis. See *Bacterium*
maduræ. See *Actinomyces*
mailei. See *Pfeifferella*
manchester. See *Shigella newcastle*
manhattan. See *Salmonella*
marcescens. See *Chromobacterium prodigiosum*
melaninogenicus. See *Fusiformis*
meleagridis. See *Salmonella*
meltensis. See *Brucella*
memphis. See *Salmonella*
minnesota. See *Salmonella*
mirabilis. See *Proteus*
mission. See *Salmonella*
mississippi. See *Salmonella*
moniliformis. See *Actinobacillus muris*
monocytogenes. See *Erysipelothrix*
montevideo. See *Salmonella*
Bacillus moribificans bovis. See *Salmonella botis-moribificans*
morgani. See *Proteus*
moskau. See *Salmonella enteritidis*
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murisepticus. See *Erysipelothrix* and *Pasteurella*
murium. See *Corynebacterium murium*
mycoides roseus. See *Chromobacterium*
neapolitanus. See *Bacterium*
neasden. See *Salmonella*
necrodentalis. See *Lactobacillus acidophilus*
necrophorus. See *Fusiformis*
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new-brunswick. See *Salmonella*
newcastle. See *Shigella*, and *Salmonella senftenberg*
newington. See *Salmonella*
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œdematoides. See *Clostridium*
onderstepoort. See *Salmonella*
oranienburg. See *Salmonella*
oregon. See *Salmonella*
ovisepticus. See *Pasteurella*
panama. See *Salmonella*
para-abortion. See *Brucella*
paracholerae. See *Vibrio*
para-influenzæ. See *Hæmophilus*
paramelitensis. See *Brucella*
parapertussis. See *Hæmophilus*
paraputrificus. See *Clostridium*
para-Shiga. See *Shigella*
parasporogenes. See *Clostridium*
parasuis. See *Brucella*
paratyphosum. See *Salmonella*
pasteurianus. See *Lactobacillus* and *Clostridium*
pensacola. See *Salmonella*
pentoaceticus. See *Lactobacillus*
perfringens. See *Clostridium welchii*
pertussis. See *Hæmophilus*
pestis. See *Pasteurella*
pestis caviæ. See *Salmonella typhi-murium*
pharr. See *Salmonella*
phlegmonis emphysematosæ. See *Clostridium welchii*

Note.—For the less common species of *Salmonella*, see pp. 823-848

suspended in 50 per cent serum. It is important to recognize that bacteria differ considerably in their resistance to phenol, staphylococci, for example, being much more resistant than the typhoid bacillus, so that in strict accuracy it is necessary to specify "typhoid phenol coefficient," "pneumococcus phenol coefficient," etc.

It is open to question as to whether compounds whose structure and chemical and germicidal activity differ greatly from that of phenol may legitimately be compared with this standard disinfectant. In recent years some workers have departed from the standard procedures, altering them to suit individual requirements. The phenol coefficient of an alcoholic solution of a compound insoluble in water obviously has little value, as has that determined on an acid or alkaline solution of a compound only slightly soluble at neutrality.³⁹ The simple statement that a given germicide has such and such a phenol coefficient has, then, little meaning.

Even assuming, however, that the standard phenol coefficient procedure be rigidly adhered to, the test itself is open to serious criticism. The end point to be determined is, clearly, sterility—all the test organisms must be destroyed. The quantitative studies discussed above have shown that although destruction may proceed at a regular and rapid rate for a time, the asymptotic tendency of the survival curve indicates that sterility or complete destruction is not a desirable end point in that it is one that is difficult to determine with a reasonable degree of accuracy. The practical consequences of the use of this end point appear in the form of aberrant results when the time variable is altered. If the end point be taken as sterility in two and one-half minutes as in the original Rideal-Walker method, the phenol coefficient of HgCl_2 is somewhat more than 2, but if the time taken is thirty minutes as in the Chick-Martin modification, HgCl_2 has a phenol coefficient of 550.⁴⁰

Furthermore, the phenol coefficient test does not take into consideration the temperature coefficient of the disinfectant under examination, which differs with the test organism,⁴¹ nor does it measure the effects of changes in concentration. A rise in temperature increases the activity of phenol and similar compounds much more than that of the salts of heavy metals; doubling the concentration of phenol increases its bactericidal activity approximately 64 times, while a similar increase in the concentration of HgCl_2 only roughly doubles its activity.

It is generally agreed that the rate of killing, *i.e.*, the reaction velocity constant, is a much more accurate measure than any value whose derivation depends upon a sterility end point. The relation between concentration of disinfectant and time required for killing is an exponential one and the reaction velocity constant, k , is given by the expression:

$$k = C^n t$$

when C is the concentration, n a constant characteristic for each disinfectant, and t is time. The temperature coefficient may, of course, be determined ex-

³⁹ For a discussion of the limitations of the phenol coefficient see Reddish: *Ind. Eng. Chem.*, 1937, 29:1044.

⁴⁰ Chick: *Jour. Hyg.*, 1908, 8:92; *ibid.*, 1910, 10:237; *ibid.*, 1912, 12:414.

⁴¹ Cf. Tilley: *Jour. Bact.*, 1942, 43:521.

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The Standardization of Disinfectants

perimentally for each disinfectant. It will be clear that this kind of characterization of the bactericidal activity of a given compound is much more informative than the usual type of phenol coefficient, no matter how precisely the conditions of the test are defined. It is not, however, generally used for routine work.⁴²

The practical value of a disinfectant is not always indicated by tests made under the controlled conditions of the laboratory. Hydrogen peroxide, for example, may give a quite respectable phenol coefficient but, when applied to an abrasion, is so rapidly decomposed under the influence of tissue catalase that its germicidal powers are almost immediately exhausted.

On the other hand, a given disinfectant may be so highly toxic for tissue cells that it has no practical value. Salle and his associates⁴³ have proposed a "toxicity index" which takes into consideration not only the germicidal activity of a compound but its toxicity for tissue as well. For example, Witlin⁴⁴ determined the concentration of bactericidal substances lethal for the chick embryo and calculated a toxicity index by using the concentration of the bactericide in grams per milliliter killing the test organism, *Staphylococcus aureus*, in ten minutes but not in five as the numerator, and the chick embryo MLD in grams for the denominator. Representative values were: phenol (1.20)—1.18, $HgCl_2$ (1.1000)—0.62, tincture of iodine—0.044, sodium hypochlorite—1.39, mercurchrome (1.50)—13.3, and values ranging as high as 9.1 for various organic mercurials. Spaulding and Bondi⁴⁵ have developed an infection-prevention toxicity test in which the tip of a mouse tail is contaminated with bacteria, the tail dipped into the solution of disinfectant, and then the tip is removed and placed in the peritoneal cavity. The highest dilution of the disinfectant protecting 50 per cent of the mice is taken as the numerator, and the greatest concentration allowing survival (from poisoning) of the test animal is taken as the denominator, to give a ratio or infection prevention-toxicity index. Such toxicity indices are of considerable practical importance in the case of skin disinfectants. There is as yet no standard method for their testing.⁴⁶

The whole question of the evaluation of disinfectants is beset with many difficulties, both theoretical and practical, and to date remains an open one to which no entirely satisfactory answer has been supplied.

⁴² For applications of this approach see, for example, Witell Jour Hyg. 1942, 42 124 339, Irwin *ibid.*, 1942, 42 328, it has also been studied extensively by Jordan and Jacobs Jour Hyg. 1944, 43 275, 363, *ibid.*, 1945, 44 210, Ann. Appl. Biol. 1945, 32 221. Jour Hyg. 1946, 44 243, 249, 421

⁴³ Salle, McOmie and Schechmeister Jour. Bact., 1939, 37 639

⁴⁴ Witlin Proc Soc. Exp. Biol. Med., 1942, 49 27

⁴⁵ Spaulding and Bondi Jour Inf. Dis., 1947, 80 194

⁴⁶ Cf. Report of the Council on Pharmacy and Chemistry Jour Amer Med Assn., 1943, 121 593.

Further light has been thrown on the probable nature of the anti-opsonic substances in aggressive exudates during the prolonged and detailed study of the factors involved in antipneumococcal immunity.

Rosenow (1907) described extracts and autolysates of virulent pneumococci which inhibited the phagocytosis of relatively avirulent pneumococci; he suggested the name *virulins* for the active substances concerned.

Cole (1917) observed that empyema fluids from pneumococcal infections contained soluble substances which specifically neutralized the protective antibodies in an antipneumococcal serum, and that similar substances might be present in the blood of rabbits suffering from experimental pneumococcal infection. He showed that, when an antipneumococcal serum was injected intravenously into a rabbit previously infected with the same type of pneumococcus, the protective antibodies disappeared very rapidly from the blood, and that a similar phenomenon could be demonstrated in patients severely ill with lobar pneumonia.

More recent studies on the antigenic components of bacterial cells (Chapter 8), and the demonstration that sensitization to the phagocytic action of polymorphonuclear cells and macrophages depends upon the union of antibody with an antigenic component situated at the surface of the bacterial cell, suggest that the substances responsible for the specific anti-opsonic effects of aggressive exudates are dissolved bacterial antigens. Landsteiner's demonstration of the inhibition of antigen-antibody reactions by simple haptens (see p 297), and the *in vitro* reactions of specific bacterial polysaccharides that fail to stimulate antibody production *in vivo*, indicate that bacterial haptens, as well as complete antigens, may be expected to exert this anti-opsonic effect.

There is good evidence that this is actually the case. Sia (1926) has studied the action of the type-specific polysaccharides of Type II and Type III pneumococci on the growth of these organisms in serum-leucocyte mixtures from the rabbit and from the cat. The pneumococci were of relatively low virulence and developed very poorly in both the serum-leucocyte mixtures employed. The addition of minute amounts of Type II polysaccharide greatly increased the growth of Type II pneumococci, but had little effect on the growth of the Type III organisms. The addition of minute amounts of Type III polysaccharide greatly increased the growth of Type III pneumococci, but not of Type II.

Felton and Bailey (1926) also demonstrated the aggressive action of Type II polysaccharide, by injecting it into mice, together with relatively avirulent pneumococci of the same type, and inducing a fatal infection. Type I polysaccharide had analogous effects (Ward 1930), and the anti-bactericidal effect proved to be due to the specific inhibition of phagocytosis (Ward and Enders 1933). The anti-phagocytic action of the Type I polysaccharide as prepared in the laboratory was not so pronounced as that of Type II and Type III polysaccharides. Nor was it as pronounced as that of extracts of pneumonic lungs infected with Type I pneumococcus (Ward 1932). The discrepancy was later resolved by the discovery that the polysaccharide preparations, unlike native polysaccharide, were not acetylated (see Chapter 8). Acetyl polysaccharide proved to be fully anti-opsonic (Enders and Wu 1934). The aggressive effect of the pneumococcal polysaccharide in human infections is suggested by the work of Cole (1917) and Park and Cooper (1928), who record that a fatal outcome in cases of lobar pneumonia in man is frequently associated with the presence in the blood of specific soluble antigen in amounts greater than can be neutralized by the antibodies that the patient has produced.

Downie (1937) observed an aggressin-like effect of Type I pneumococcal polysaccharide. Mice immunized with a single dose of polysaccharide were resistant to a given dose of Type I pneumococci. When this dose was injected together with 0.05 mgm. of the same polysaccharide, the immunity was in most cases abolished. Nye and Harris

BACTERIAL HEREDITY AND VARIATION

Before the development of methods of isolating bacteria in pure culture many observers regarded these organisms as members of a markedly homogeneous group, possibly of but a single species, which showed a high degree of morphological variation. The interconversion of coccus, bacillary and spiral forms was considered to be of common occurrence and, in consequence, the observed morphological differences among the bacteria were held to be of no significance. This doctrine, designated as *pleomorphism*, reached its height in 1877 with Nageli, who postulated but a single species to which all bacteria belonged. It should be noted that the term *pleomorphism* included not only morphological variation but equally facile alterations in virulence; the innocuous hay bacillus (*Bacillus subtilis*), for example, presumably could change suddenly into the highly virulent anthrax bacillus.

The development of pure culture techniques by Koch and others swung the pendulum to the other extreme, and the doctrine of *monomorphism* became predominant. It was held by Koch and other extremists, whose camp the great majority of bacteriologists soon joined, that a given bacterial species existed in one and only one form, and that aberrant forms were either evidence of contamination of the pure culture or were the so-called involution or degenerative forms which were dead or dying and therefore had no significance. This *monomorphism* was confined largely to bacterial form and, although abrupt changes such as the conversion of the hay bacillus to the anthrax bacillus were denied, alteration in the virulence of pathogenic bacteria was not regarded as inconsistent with an absolute constancy of form.

Despite the currency of this extreme *monomorphism*, evidence began to accumulate which indicated that, though most bacteria showed a remarkable constancy of form, morphological and physiological variation of these organisms is not uncommon. The last thirty or forty years have seen the development of an extensive literature on this subject. Much of the evidence which has accumulated is of just sufficiently inconclusive character that widely different interpretations may be made. Contemporary workers fall into two groups in this respect—the conservatives, a group which includes the majority of workers, who still adhere to the doctrines of Koch and his school in a modified form and who accept the well established variations as observed facts whose full significance is as yet not apparent, and the radicals, who profess to see evidences of complex life cycles, conjugation, sexual reproduction and kindred phenomena in the reported observations. It is not improbable that a sound interpretation lies somewhere between these views.

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Bacterial Heredity and Variation

The biological significance of observed variations and their relation to similar phenomena occurring in the higher forms of life are difficult to assess. The difficulties stem in part from a strong tendency to regard bacteria as somehow "different" from other living organisms. This inherently sterile concept has arisen partly because of the apparent absence of a morphologically discrete nucleus in the bacterial cell, partly because the characteristics of bacteria which vary are generally not directly comparable to the genetically determined characters which are studied in the higher organisms, and partly because the bacteria are subject to the influence of unique environmental factors.

While it seems not unlikely that the chromatinic bodies described by Robinow (p. 51) represent an organized nucleus in the bacterial cell, there is as yet no indication of an intranuclear differentiation into structures analogous to chromosomes. Present evidence suggests that nuclear division, if the division of chromatinic bodies may be so regarded, is not as complex, at least morphologically, as the process of mitosis. While there is evidence, as will appear, of a spatial orientation of hereditary determinants, no corresponding morphology is as yet apparent, and the cytological techniques so essential to the understanding of the genetics of higher organisms have not been applicable to the bacteria.

The differential characters which serve to distinguish bacteria from one another are, in general, of a greater degree of fineness than those which the biologist is accustomed to use. The bacteriologist is concerned with characters not so gross as the red and white eyes of *Drosophila* but which might be considered analogous to the individual enzyme systems operative in the formation of red and white eyes. When such characters have no counterpart among those known to be of fundamental significance to the higher organism, and most if not all of them as yet have not, it is extremely difficult to judge whether they are of deep-seated importance to the cell or whether they are biologically trivial. Loss of virulence by a pathogenic organism, while of great practical importance to its prospective host, may be of only minor significance to the bacterium. Analogies are, then, difficult to draw and are always of doubtful validity.

Not only are the environmental factors which affect the bacterial cell to some degree peculiar to unicellular organisms of minute dimensions, but the essential obscurity of the bacterial environment is, to no small degree, a consequence of the limits of human imagination. Some aspects of this bacterial environment are known. For example, the tremendously exaggerated effect of surface tension on objects as small as bacteria must profoundly influence the bacterial form. The continuous molecular bombardment to which these organisms are subject and to which, because of their small size, they respond with the dancing motion of brownian movement, may be a factor which influences their structure and rigidity of form. The predominance of interfacial phenomena in the bacterial world of near-molecular dimensions undoubtedly contributes to its general character. There is evidence, for example, that hydrogen ion activity and reducing intensities may be quite different at interfaces from what the observer of gross phenomena assumes them to be. The environment to which these and undoubtedly other phenomena

character is, clearly, an abstract one, and the interpretation of morphological or physiological changes taking place under such circumstances is, to say the least, difficult.

From these considerations it is easy to understand how bacteria can come to be regarded as "different" without real justification for such a belief. It cannot, then, be emphasized too strongly that, so far as is known, bacteria do not differ in any essential way from other living cells and any interpretation of variation or other phenomena must rest on a sound biological foundation.

Before discussing the kinds of bacterial variation which are observed, two concepts fundamental to the general problem must be considered. One of the most important of these is that of the "normal" morphological or physiological state of a bacterium. The anthropocentric tendencies of the human mind

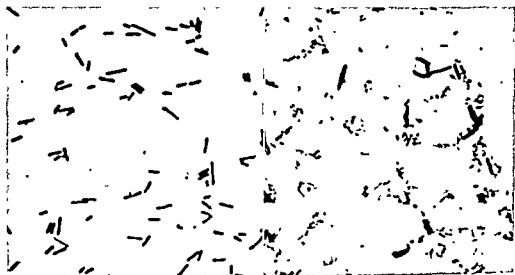


Fig. 24. The morphology of *Rhizobium*. Left, a smear from a pure culture on laboratory media; note the regularity of the "normal" bacillary morphology. Right, smear from a clover root nodule showing swollen, branched and coccoid forms, the so called bacteroides Fuchs, $\times 1050$.

stimulate the belief that the normal form and functions of a bacterium are those which are manifest in culture on the ordinary laboratory media. Certain evidence suggests, however, that the term normal is but a relative one. The symbiotic nitrogen-fixing organisms show a relatively constant "normal" morphology on the usual laboratory media, but in the root nodule they are found in a variety of bizarre forms designated as bacteroides (Fig. 24). Which, then, may be considered to be the "normal" form, that observed in the laboratory or that shown by the organisms in what might be regarded as their natural habitat? The physiological differences between bacteria in pure culture and those in the mixed cultures occurring in nature have been discussed in Chapter 4. Clearly, then, the term normal, although an extremely useful one, cannot be taken to have real meaning.

The second concept is that of bacteria in terms of populations. The existence of bacteria as population groups composed of enormous numbers of individuals and a rapid rate of cell division results in a compression in both time and space of biological processes. The resulting exaggeration of variabil-

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Bacterial Dissociation

ity of the bacteria as compared to that of other living organisms, coupled with the selective effect of population pressure as numbers approach a limiting density, undoubtedly contributes in very large part to the apparent flexibility in the response of bacteria to environmental conditions. The importance of the population aspect of bacteria has only recently become more generally recognized,¹ though inter- and intraspecies relationships at the population level of other organisms constitute a large part of ecology.

Here bacterial variation will be considered under two general heads, the kinds of variation observed, and the mechanisms operative in such variation.

OBSERVED VARIATIONS OF BACTERIA

Bacteria have been found to vary, sometimes widely, in all the characteristics made use of in their differentiation and identification. These include morphology, both macroscopic and microscopic, physiological properties, including ability to produce disease; and immunological character. Changes may appear suddenly, i.e., in a single subculture, or they may become apparent only gradually as in aged cultures or over many transplants or animal passages and some hundreds of generations. The rapidity of appearance, however, cannot be taken to have fundamental significance and, in fact, in many instances is subject to experimental manipulation. Variants may appear apparently spontaneously, or may be seemingly induced by making the culture medium mildly and specifically toxic, as by the inclusion of lithium chloride, antiserum, antibacterial substances and the like, or by making it selective so that the expected variant is given enhanced survival value as, for example, by including a sugar in the medium in attempts to isolate fermenting variants of a non-fermenting parent strain. The relative importance of the characters subject to variation is by no means clear, and some may be biologically trivial while others are a reflection of deep-seated changes within the cell. It may be emphasized that relative biological importance is not necessarily related to practical importance.

MORPHOLOGICAL VARIATION

Variation in the morphology of bacteria may be considered under two general heads, colonial morphology or that of masses of cells, and the morphology of individual bacterial cells.

Bacterial Dissociation. A remarkable type of bacterial variation, whose most obvious outward manifestations is a change in the type of colony formed on semi-solid media, was observed by Baerthlein in Germany (1918). Adwight in England (1921) and de Kruif in the United States (1921). The phenomenon was termed by de Kruif bacterial or microbial dissociation, a term which has, in spite of certain undesirable features, been generally adopted. The ordinary laboratory culture, particularly old broth cultures, when plated out on an agar medium develops into two kinds of colonies; one, smooth (S), round, convex and shining; the other, rough (R), irregular, flattened and wrinkled. In addition to these obviously different extreme colony types (Fig. 25), all degrees of intermediate (SR and RS) types may usually be found. The transformation of the S form, usually regarded as the

¹ See Braun, *Bact. Rev.* 1947, 11-75.

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cultures by Braun.² Similarly, the inclusion of antiserum to one dissociative form markedly favors the development of the heterologous form.

The G Colony. Another type of colony, the so-called G type, although apparently seen by earlier observers in 1910 and 1911, was brought to general notice by Hadley and his co-workers. These colonies are very small, 0.05 mm. or less in diameter, and appear to be made up of small cells of diverse morphology. They may be regarded as variants with an inherently lowered metabolic rate which perhaps permits survival under unfavorable conditions.³

L Variation. Perhaps related to G colonial variation is the so-called L variation. The normal mode of reproduction of the pleuropneumonia-like organisms and the non-sporulating obligate anaerobic bacteroides includes the formation of greatly swollen cells or large spherical bodies from which bacilli or granular forms are liberated (pp. 540 and 548). The latter give rise to very minute colonies similar to the G colony. This phenomenon has also been reported as occurring in other bacteria such as *Proteus*, but with somewhat less convincing evidence.

Variation in Cell Structures. As indicated above, colonial morphology is in part a function of the morphology of the individual cells making up the colony, and one of the most important factors in the differential morphology of the S and R variants is the occurrence of hydrophilic polysaccharide on the surface of the cell. As pointed out elsewhere (p. 42), this frequently takes the form of a layer or morphologically demonstrable capsule on the outer surface of the cell.

Capsules. Capsule formation or, from the physiological point of view, the synthesis of capsular substance usually of polysaccharide but sometimes of polypeptide nature, is very common among the bacteria, but a morphologically demonstrable capsule is not always apparent. Capsule formation is dependent in large part on the environment, and the most favorable medium in the case of pathogenic bacteria in which capsule formation is associated with virulence is the body of the susceptible animal. The anthrax bacillus forms a heavy capsule in the infected animal, but there is little or no evidence of a capsule when the bacilli are cultivated on laboratory media. The pneumococcus, likewise heavily encapsulated in the infected animal, also forms a capsule in artificial media, but the capsule is best developed when the medium is enriched with blood or serum and carbohydrate. Among the pathogenic forms, capsule formation tends to diminish with continued cultivation on artificial media, probably because these furnish a somewhat less than optimal environment, and is restored by animal passage of the bacteria.

The heavily encapsulated saprophytic bacteria, such as the dextran- and levulan-forming cocci of the genus *Leuconostoc*, show no tendency to loss of capsule formation in culture provided that carbohydrate is supplied, and it seems probable that the artificial medium is more nearly optimal than it is in the case of the more fastidious pathogenic bacteria. In general, then, capsule formation is determined in large part by the environment, may be a quantitative matter and is reversible.

Capsule formation may be specifically inhibited, however, the most effective

² Braun. *Jour. Bact.*, 1946, 51:327

³ See Colwell: *Jour. Bact.*, 1946, 52:417.

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Involution Forms

tive means being the inclusion of antiserum to the capsular material in the culture medium. The loss of capsule formation under such circumstances, or when it is relatively complete by thorough prolonged culture on artificial media, results in the S-R dissociation, and the lack of a capsule is characteristic of the rough variant. As indicated above, this loss is also reversible, though frequently only with considerable difficulty. Whether it is to be regarded as basically different from a gradual diminution in the amounts of capsular substance synthesized through continued cultivation in a less than optimal environment is not clear. The relatively rapid appearance of non-encapsulated variants may be a consequence of the highly selective effect of antiserum rather than indicative of a mutation-like change.

Flagella. The occurrence of flagella on motile bacteria is also subject to variation. There appears to be little or no variation with respect to numbers or position of flagella, but their presence or absence is variable. Non-motile variants of motile bacteria are occasionally, though not commonly, observed, and these appear to be stable forms with little tendency to reversion to the motile form. In the case of very actively motile bacteria such as *Proteus*, colonial morphology is affected by motility in that the motile bacteria "swarm" and the growth spreads in a thin film over the surface of an agar medium, while the non-motile variants form discrete colonies.

The formation of flagella is subject to environmental influence also. Actively motile species kept in stock culture on laboratory media tend to lose their motility, and potentially motile bacteria may be motile when cultivated at one temperature but not at another. The inclusion of antibacterial substances such as phenol in the medium in toxic but non-lethal concentrations inhibits the formation of flagella by bacilli of the *Salmonella* group, in fact, one method of obtaining these forms free from flagella and the antigenic substances associated with them is culture on nutrient agar containing 0.1 per cent phenol.

Spores. The spore is a consequence of the aggregation of cell substance within a spore case during spore formation. It is this process rather than the formation of a structure of the vegetative cell that is subject to variation. Like capsule formation it is essentially a physiological process but with morphological consequences. Spore formation is affected by environmental factors, and asporogenous variants which breed true also occur. In the case of the anthrax bacillus, for example, spores are formed only when the bacteria have access to free oxygen, only vegetative forms are found in the infected animal until the bacilli are exposed to air by tissue decomposition or autopsy and then spore formation occurs. Similarly, spores are formed most rapidly at 32° C., less so at 37° C., and not at all when the culture is incubated at 42° C. The inhibition of spore formation by the anthrax bacillus by high temperatures is a temporary one in that when the temperature of incubation is reduced spore formation again occurs, but if cultures are maintained at 42° C. for many transfers the ability to form spores is apparently permanently lost. In general, however, spore formation is a relatively stable character.

Involution Forms. The occurrence of aberrant or "abnormal" forms of bacteria is very common and observed most frequently in old cultures. As indicated earlier (p. 40), such forms are commonly regarded as involution

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forms, that is to say, dead or dying cells, and it has been pointed out that the physical structure of dead bacteria breaks down, with the protoplasm becoming granular and escaping into the surrounding medium with disintegration of the cell wall (Fig. 5). In addition to such dead cells, filamentous and coccoid forms of bacilli, swollen cocci, and the like are found with some frequency.

The tendency to aberrant morphology is in part a function of the structural rigidity of the bacterial cell, and in part an effect of the environment on the cell. It is self-evident that this should be so in a macromolecular environment; for example, no small degree of structural rigidity is necessary to maintain a bacillary form in the face of the forces of surface tension, and any crumbling of that structure will of necessity result in misshapen cells.

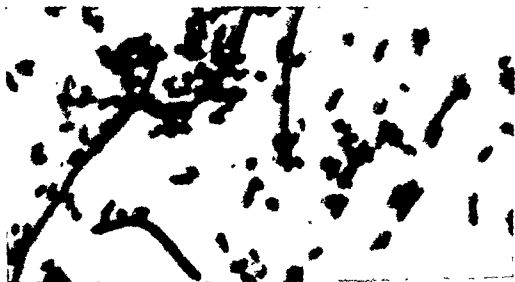


Fig. 26. Involution forms of the typhoid bacillus; fuchsin stain. Note the filamentous forms and elongated rods mixed with the typical forms. What appear to be buds on the filament are probably adjacent cells. The poor resolution at these high magnifications is apparent. $\times 3500$.

Thus, the non-sporulating anaerobic bacilli (p. 540) and the pleuropneumonia-like organisms (p. 547) are perhaps the most fragile of all bacteria, and in keeping with this have a highly variable morphology. Similarly, the cholera vibrio is a fragile bacterium, easily broken up by grinding procedures, and is notorious for its morphological instability.

In old cultures accumulated metabolic products not only affect the viability and reproduction of cells, but may well contribute more directly to the development of aberrant forms. Morphological variation may be produced experimentally by manipulation of the medium. If, for example, surface tension is lowered by the inclusion of a surface tension depressant, aberrant morphology results, and among the bacilli the rod form becomes elongated to give rise to filaments. Analogous effects are produced by the cultivation of bacteria in the presence of toxic but not lethal concentrations of salt. Another kind of effect is produced by the inclusion of antibiotic agents which inhibit cell division; the cells continue to metabolize but fail to divide, and assume swollen, misshapen forms.

It will be clear from the foregoing that a large proportion of morphologic-

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ally abnormal bacterial cells are to be found in old cultures which arise as a consequence of the toxicity of accumulated metabolic products, and the disintegration of the structure of dead and dying cells. It is not so clear to what extent essentially normal physiological activity and ability to reproduce are associated with the structural integrity of the cell. Certainly some degree of distortion is not fatal, for example, the filamentous forms of bacilli produced by depression of surface tension of the medium are viable in many cases. It seems probable, however, that the physical organization of the cell is of no small importance, and in this connection it is significant that cultural studies of the aberrant forms found in old cultures have indicated that in the great majority of instances these forms are dead in that they are incapable of multiplication. A part, perhaps a very large part, then, of variation in the morphology of the bacterial cell is no more than the outward evidence of a dying population and the death and disintegration of individual cells.

Bacterial Life Cycles. Cyclical development of bacteria in a succession of morphological types is well known in some cases. The simplest is the spore-vegetative cell succession of the sporulating bacteria. The succession of morphological types, or cytomorphosis (p. 59), associated with the growth of the bacterial population discussed earlier, and the successive zoogeal and monad or swarmer stages of *Nitrobacter* also seem well established. Considerably more complex developmental cycles, or cyclogenies, analogous to those well known among the fungi and higher plants, have been postulated by some workers for many of the well-known bacteria such as the staphylococci, streptococci, enteric bacilli and the like.

The experimental evidence upon which the belief in bacterial life cycles is based consists of observations of aberrant, "abnormal" forms occurring in pure cultures. It is postulated that the so-called "normal" bacterial form is but one morphological stage of many in which a bacterium may exist but in which it is temporarily fixed by the constant environment of the laboratory medium. The appearance of aberrant forms is, then, evidence of a tendency on the part of the organism to assume the forms characterizing other stages of the life cycle, and such forms should not be termed involution or degenerative forms. The aberrant cells in turn break down to liberate minute viable organisms either directly or via a symplastic stage. The minute forms, designated as gonidia, gametes, microgametes and the like, may give rise to the "normal" vegetative cell, either directly or after a process of conjugation or sexual union.

In any attempt to weigh the evidence in favor of bacterial life cycles two points must be borne in mind. In the first place, the aberrant forms which constitute an essential part of the life cycle occur only in the death phase of a bacterial culture, never during the phases of active growth; and, in the second, many of the postulated forms are too small to permit resolution with visible light. Alternative explanations of aberrant morphology are many. Suppose the cell dies, autolysis follows with the breakdown of cell constituents to smaller molecules, the increase in the number of molecules in solution raises the osmotic pressure within the cell and the cell imbibes water with resultant distention and eventual disruption. Granular material may be volutin and similar granular substances commonly observed in bacterial cells.

It is perhaps significant that studies utilizing the micro motion picture tech-

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nique for the continuous observation of the development of bacteria from a single cell have yielded no evidence of a bacterial life cycle.⁴ The evidence for the existence of such life cycles is exceedingly tenuous for two reasons. Much of it is open to serious technical criticism,⁵ and in all cases there are equally plausible alternative explanations. Aside, then, from the point of technical criticism, differences in opinion are largely a matter of interpretation and, in the absence of conclusive experimental evidence, which has as yet not been forthcoming, are likely to remain so for the time being.

Filterable Forms of Bacteria.⁶ Perhaps associated with bacterial life cycles are the so-called filterable forms of these organisms. The visible, readily cultivable forms of bacteria such as are present in the ordinary "pure culture" do not usually pass through the finer-pored porcelain and infusorial earth filters. That there are particular conditions, however, that favor the transmissibility of certain bacteria through these filters has become increasingly evident. Aside from the technical difficulties that beset all filtration experiments there are serious divergences of interpretation. Many observers have reported that familiar microorganisms such as the tubercle bacillus and the typhoid bacillus can sometimes pass through well constructed filters and this they interpret as signifying the existence of a filterable stage in the life history of these bacteria. It is even alleged by some that the filterable viruses of smallpox, poliomyelitis and other diseases represent a filterable phase in the life cycle of visible microorganisms, streptococci or what not, a view that has been urged especially by Rosenow⁷ in this country and Enderlein⁸ in Europe. This view is by no means generally accepted.

On the other hand, it is maintained that the so-called "filterable" forms of the typhoid bacillus, for example, are merely cells dwarfed by inadequate nutrition or are viable cell particles so small as to pass through filters but with capacity of renewed growth when again placed in favorable surroundings. It may be urged that the ability of cell fragments to regenerate is no new thing in biology, and it is quite as plausible to regard minute filterable forms of cocci or tubercle bacilli as portions of fragmented cells as to look on them as representing a significant filterable phase in their life histories.

PHYSIOLOGICAL VARIATION

Variation in the physiological activities of bacteria is exceedingly common. It may take various forms such as alterations in virulence, *i.e.*, ability to produce disease, changes in fermentative activity and nutritive requirements, or the acquisition of resistance to antibacterial agents.

Attenuation. The term attenuation is ordinarily loosely used to mean reduced virulence of a pathogenic microorganism. It may indicate a simple loss of ability to produce disease in a general sense, or a reduction in virulence for one host species accompanied by an increase in virulence for another host species.

⁴ Wyckoff: *Jour. Exp. Med.*, 1934, 59:381.

⁵ For example, see Holman and Carson: *Jour. Inf. Dis.*, 1935, 56:165; also Lamanna: *Jour. Bact.*, 1944, 47:327.

⁶ Hadley, Delves and Klimak: *Jour. Inf. Dis.*, 1931, 48:1.

⁷ For example, see Rosenow: *Amer. Jour. Clin. Path.*, 1944, 14:150.

⁸ Enderlein: *Arch. Entwicklungsgesch. Bakt.*, 1940, 1:252.

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In general most pathogenic bacteria tend to lose virulence when kept in culture on artificial media. For example, pneumococci rapidly lose virulence for the mouse and in even a very few transfers on enriched media the minimal lethal dose increases by several hundred fold. Staphylococci, however, retain their virulence over many transplants on artificial media, but eventually become relatively avirulent. A few bacteria, such as the anthrax bacillus, seem to retain virulence almost indefinitely but this is not common. Such losses in virulence are general in character in that the bacteria become less virulent for a variety of experimental animals, and presumably arise as a consequence of adaptation to an environment other than that of the animal body. The artificial environment may be deliberately made somewhat toxic in order to bring about such changes. Simple aging in the case of Pasteur's cultures of the fowl cholera organism was sufficient to reduce virulence so that a fatal infection was not produced, and the attenuated strain of bovine tubercle bacillus known as BCG was carried on a bile-containing medium until its virulence was apparently completely lost.

Loss of virulence may be accompanied by changes in other characteristics such as marked diminution in capsule formation by the pneumococcus, a loss of golden pigment by *Staphylococcus aureus*, somewhat less fastidious nutritive requirements, etc. In many instances the change is dissociative in nature and loss in virulence is associated with a shift from the smooth form. In most bacteria, of course, virulence is markedly reduced by the S-R dissociation, and a change of this kind may occur *in vivo*, presumably under the influence of antibody; for example, the typhoid bacilli excreted by most chronic carriers are rough, avirulent forms.

Virulence may often be restored by animal passage, e.g., successive and repeated infection and reisolation of the bacterium through a series of experimental animals. It cannot be concluded that the microorganisms are individually altered in the restoration of virulence by animal passage, and it is more probable that the animal acts as a highly selective screen, separating out those few bacteria in the inoculum which multiply most rapidly in the tissues. The restoration of virulence is usually accompanied by a restoration of other characters associated with it such as capsule formation.

The virulence of a microorganism may be modified by passage through another host species and sometimes, though not invariably, virulence for the original host species is reduced. The classic example of attenuation by animal passage is that of Pasteur's attenuation of the rabies virus by adaptation to the rabbit brain. The original strain from dogs, "street virus," kills rabbits in about two weeks following subdural inoculation, after some twenty passages it kills in eight days, and after an additional twenty or more passages the period may be reduced to seven days but cannot be reduced further. This "fixed virus" has acquired such a marked affinity for the nervous tissue that it will not produce rabies on subcutaneous inoculation and so may be used as an immunizing agent. Such attenuation or adaptation to a new host is very common among the viruses, and includes the adaptation of a variety of viruses to the chick embryo, the adaptation of yellow fever virus to the mouse brain, etc. Perhaps the best known single example is that of the conversion of smallpox virus to cowpox virus.

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Attenuation of virulence is of considerable practical importance in relation to the preparation of immunizing agents. Among the bacterial diseases, killed vaccines are not effective immunizing agents in anthrax and tuberculosis, but attenuated anthrax bacilli were originally used by Pasteur to immunize sheep, and the attenuated strain of tubercle bacilli, BCG, has been of increasing interest as an immunizing agent in man. Among the virus diseases, effective immunity is rarely obtained with killed material, and vaccines of attenuated virus, often given in conjunction with antiserum, are very valuable.

Biochemical Variation. Variation in the biochemical properties of bacteria, such as fermentation of sugars, decomposition of proteins and amino acids, nutritional requirements, resistance to antibacterial agents, and the like occurs with some frequency. Changes may appear seemingly spontaneously, but more often seem to be in the nature of an adaptive response to the environment.



Fig. 27 Colonies of *Bact. coli mutabile* on lactose agar. Note the lactose-fermenting papillae of the variant appearing on the non-lactose-fermenting colonies (Parr).

Mutation-like Variation. The best known example of variation of this kind was first observed by Massini in 1907. He isolated a non-lactose-fermenting strain of *Bacterium coli* which, when cultivated on lactose agar containing an indicator which turned red in the presence of acid, gave rise to white colonies. On continued incubation, however, red papillae appeared on the colonies, indicating that some of the bacteria were decomposing the sugar to acids. This is illustrated in Fig. 27. Subcultures from the red papillae bred true and showed no tendency to revert to the non-lactose-fermenting form, while subcultures from the white portions of the colonies gave rise to white colonies on which red papillae appeared as before. He concluded that the change was a mutation and that some strains of the colon bacillus, which he named *Bacterium coli mutabile*, were genetically unstable with a tendency to throw off lactose-forming mutants. Such strains of colon bacilli have since been found repeatedly and the variation has been studied in some detail. It was found by Lewis⁹ that if such strains were cultured on a synthetic medium containing lactose as the only source of organic carbon, a constant proportion,

⁹ Lewis Jour. Bact., 1934, 28 619.

Further light has been thrown on the probable nature of the anti-opsonic substances in aggressive exudates during the prolonged and detailed study of the factors involved in antipneumococcal immunity.

Rosenow (1907) described extracts and autolysates of virulent pneumococci which inhibited the phagocytosis of relatively avirulent pneumococci; he suggested the name *virulins* for the active substances concerned.

Cole (1917) observed that empyema fluids from pneumococcal infections contained soluble substances which specifically neutralized the protective antibodies in an antipneumococcal serum, and that similar substances might be present in the blood of rabbits suffering from experimental pneumococcal infection. He showed that, when an antipneumococcal serum was injected intravenously into a rabbit previously infected with the same type of pneumococcus, the protective antibodies disappeared very rapidly from the blood, and that a similar phenomenon could be demonstrated in patients severely ill with lobar pneumonia.

More recent studies on the antigenic components of bacterial cells (Chapter 8), and the demonstration that sensitization to the phagocytic action of polymorphonuclear cells and macrophages depends upon the union of antibody with an antigenic component situated at the surface of the bacterial cell, suggest that the substances responsible for the specific anti-opsonic effects of aggressive exudates are dissolved bacterial antigens. Landsteiner's demonstration of the inhibition of antigen-antibody reactions by simple haptens (see p. 297), and the *in vitro* reactions of specific bacterial polysaccharides that fail to stimulate antibody production *in vivo*, indicate that bacterial haptens, as well as complete antigens, may be expected to exert this anti-opsonic effect.

There is good evidence that this is actually the case. Sia (1926) has studied the action of the type-specific polysaccharides of Type II and Type III pneumococci on the growth of these organisms in serum-leucocyte mixtures from the rabbit and from the cat. The pneumococci were of relatively low virulence and developed very poorly in both the serum-leucocyte mixtures employed. The addition of minute amounts of Type II polysaccharide greatly increased the growth of Type II pneumococci, but had little effect on the growth of the Type III organisms. The addition of minute amounts of Type III polysaccharide greatly increased the growth of Type III pneumococci, but not of Type II.

Felton and Bailey (1926) also demonstrated the aggressive action of Type II polysaccharide, by injecting it into mice, together with relatively avirulent pneumococci of the same type, and inducing a fatal infection. Type I polysaccharide had analogous effects (Ward 1930), and the anti-bactericidal effect proved to be due to the specific inhibition of phagocytosis (Ward and Enders 1933). The anti-phagocytic action of the Type I polysaccharide as prepared in the laboratory was not so pronounced as that of Type II and Type III polysaccharides. Nor was it as pronounced as that of extracts of pneumonic lungs infected with Type I pneumococcus (Ward 1932). The discrepancy was later resolved by the discovery that the polysaccharide preparations, unlike native polysaccharide, were not acetylated (see Chapter 8). Acetyl polysaccharide proved to be fully anti-opsonic (Enders and Wu 1934). The aggressive effect of the pneumococcal polysaccharide in human infections is suggested by the work of Cole (1917) and Park and Cooper (1928), who record that a fatal outcome in cases of lobar pneumonia in man is frequently associated with the presence in the blood of specific soluble antigen in amounts greater than can be neutralized by the antibodies that the patient has produced.

Downie (1937) observed an aggressin-like effect of Type I pneumococcal polysaccharide. Mice immunized with a single dose of polysaccharide were resistant to a given dose of Type I pneumococci. When this dose was injected together with 0.05 mgm. of the same polysaccharide, the immunity was in most cases abolished. Nye and Harris

about one in 100,000, of the cells were able to grow. Further studies by Deere¹⁰ have shown that both fermenting and non-fermenting varieties contain the enzymes necessary for the lactose fermentation, and suggested that the fermenting variants differ from the parent strain in that the cell wall is permeable to lactose.

A similar mutation-like change with respect to citrate utilization by coliform bacteria has been observed by Parr and Simpson,¹¹ and extensive experiments with other members of the colon-typhoid-dysentery group of bacilli have shown that this kind of variation with respect to the fermentation of other substances such as dulcitol is not uncommon. A number of these organisms will, when cultivated on media containing a sugar they are unable to ferment, respond with the formation of such papillae, the tendency toward reversion to the parent type varying from one organism to another.

Mutation-like variation occurs in properties other than sugar fermentation. One of the most adequately studied of these is pigment production by *Bacterium prodigiosum*, which has been investigated by a number of workers, especially Bunting.¹² During growth of the culture white, pale pink, bright pink and dark red variants are thrown off at a relatively constant rate, one per 10,000 cells in the case of dark red and one per 3000 cells in that of bright pink, but, with rare exceptions in the case of white and pale pink, these variants do not breed true and on subculture show essentially the same color distribution as the parent strain.

Variation by Adaptation or Training. Changes in biochemical properties also occur in a seemingly more gradual way, induced by continued cultivation in appropriate media. Such variation in nutritive requirements is the basis for the general observation that the more fastidious pathogenic bacteria are frequently more difficult to cultivate on primary isolation and enriched media are required, while, after being carried in culture for some time, they grow more rapidly and profusely and on somewhat simpler media. For example, on primary isolation *Brucella abortus* requires an increased carbon dioxide tension, but after a few subcultures this may be dispensed with. Similarly, the gonococcus and meningococcus require, on primary isolation, both an increased carbon dioxide tension and an enriched medium, usually a heated blood medium. After a few subcultures, however, it is no longer necessary to supply carbon dioxide, and eventually growth occurs in simpler media.

This process of adaptation to growth on an originally deficient medium has been studied with respect to specific nutritional requirements. Fildes, Gladstone and Knight¹³ found, for example, that some strains of the typhoid bacillus which require tryptophane could be trained to grow without this amino acid by successive culture in decreasing concentrations of it. Gladstone¹⁴ similarly showed that some strains of *Staphylococcus aureus* that initially required a number of amino acids could eventually be adapted to grow in the presence of an ammonium salt as the only source of nitrogen, and

¹⁰ Deere Jour. Bact., 1939, 37 355, 473.

¹¹ Parr and Simpson Jour. Bact., 1940, 40 467.

¹² Bunting Jour. Bact., 1940, 40 57, 69, *ibid.*, 1942, 43 555, 593, summarized Somp. Quant. Biol., 1946, 11 25.

¹³ Fildes, Gladstone and Knight Brit. Jour. Exp. Path., 1933, 14 189.

¹⁴ Gladstone Brit. Jour. Exp. Path., 1937, 18 322.

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Koser and Wright¹⁵ could adapt dysentery bacilli requiring nicotinic acid to growth in its absence. Ability to ferment carbohydrates may also be either acquired or markedly enhanced by continued cultivation in the presence of the substrate or by very heavy inoculation of highly specific media in which the substrate is the only nutritive material. Still other adaptations such as to changes in pH, increased incubation temperature and the like also occur, but are usually not marked.

*Adaptive Enzymes.*¹⁶ The ability of a microorganism to decompose a substrate may be very rapidly enhanced in the presence of that substrate. Although known for many years, this particular kind of bacterial variation has been the subject of renewed interest as *enzyme adaptation*. The enzymes which may be formed under the influence of the presence of substrate have been called adaptive enzymes in contrast with those which are formed whether or not the substrate is present, the constitutive enzymes. The formation of adaptive enzymes may be demonstrated by cultivation of the bacteria in a medium containing the substrate and then testing the organisms for enzymatic activity, or a washed suspension of bacteria grown in the absence of the substrate may be mixed and incubated with it. In the first instance, activity is immediately apparent, the bacteria having formed the adaptive enzyme during growth, and in the second a latent period varying from thirty minutes to two to four hours occurs before rapid decomposition of the substrate begins.

Thus, washed suspensions of the colon bacillus bring about an equally rapid decomposition of glucose, as measured by oxygen uptake in respirometers, regardless of whether the medium upon which the organisms were grown contained this sugar. On the other hand, the enzyme system of this organism which is responsible for the decomposition of tryptophane to indol is an adaptive enzyme. If the medium upon which they are grown contained tryptophane, washed suspensions immediately convert the amino acid to indol, but if the medium did not contain tryptophane and the washed bacilli are suspended in tryptophane solution, decomposition to indol occurs only after a latent period. Similarly, the amino acid decarboxylases are formed for the most part in the presence of the substrates. Many of the bacterial proteases are adaptive enzymes, for only small amounts of the enzymes are formed in the absence of protein, but filtrates from cultures in protein-containing media are actively proteolytic. Various other bacterial enzymes are adaptive also, thus, the enzyme responsible for the reduction of tetrathionate to thiosulfate by *Salmonella paratyphi* B, tetrathionase, and that catalyzing the reduction of nitrate to nitrite by *Bact. coli*, nitratase, are adaptive.

The question of whether or not the formation of an adaptive enzyme is associated with bacterial multiplication is one of considerable interest. In general it appears that some degree of multiplication is essential (cell division occurs even in suspensions of washed bacteria), and it has been shown in the formation of galactozymase by *Bact. coli* that the amount of enzyme was proportional to the number of new cells.¹⁷ In a few instances, however, adaptive

¹⁵ Koser and Wright: Jour. Bact., 1943, 46:239.

¹⁶ For general discussions see Karstrom: Ergeb. d. Enzymforsch., 1938, 7:350, Dubos: Bact. Rev., 1940, 4:1; Gale: Bact. Rev., 1943, 7:139.

¹⁷ Stephenson and Gale: Biochem. Jour., 1937, 31:1311.

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Note.—For species of *Salmonella* not given in this list, see pp. 823-848

enzymes have been found to be formed by washed bacteria in the presence of the substrate and under circumstances in which there was no detectable cell division; these include the formic hydrogenylase of *Bact. coli*,¹⁸ the galactozymase of *Saccharomyces cerevisiae*¹⁹ and the nitratase and tetrathionase of *Salmonella paratyphi* B and *Bact. coli*.²⁰ The formation of adaptive enzymes by partially poisoned bacteria, i.e., those which cannot reproduce but continue to metabolize, has, however, not been observed.

The distinction between adaptive and constitutive enzymes is not a sharp one, for frequently, as in the case of the bacterial proteases, the adaptive enzyme is not completely lacking in the absence of the substrate. It is clear that there is some relation between the formation of adaptive enzymes and adaptation to a substrate by serial culture in its presence in that the distinction appears to be in part a quantitative one. In bacterial growth enzyme adaptation is expressed in terms of the lag period and the generation time during the period of active growth. What might be called the population-growth expression of adaptive processes has been studied at some length by Hinshelwood and his colleagues.²¹ They have shown that, in the adaptation of *Bact. aerogenes* to the most efficient utilization of substrates such as glycerol, disaccharides, glycine, etc., the adaptation is expressed as a transition from a slower to a more rapid rate of growth in the early stages as indicated by breaks in the growth curves, and as a decrease in mean generation time in the period of logarithmic growth, the latter decreasing from 70 to 110 minutes for unadapted strains to 32 to 33 minutes for adapted ones. The process of adaptation cannot be fully explained, however, on the basis of rate of formation of adaptive enzymes, for an adaptive enzyme, such as a bacterial protease, does not tend to persist as a constitutive enzyme when the bacterium is kept on protein-containing media for many transfers and then cultured in the absence of protein. An adapted strain tends to breed true to a certain extent in that there is a tendency for the acquired property to persist in the absence of the substrate, and the tendency is directly related to the extent to which adaptation is carried. Strains of bacteria vary, but in a general sense it might be said that following fifteen transfers in substrate-containing media, the property tends to disappear at about the same rate as that at which it was acquired, but if the process of adaptation has been carried on for, say thirty transfers, it may persist indefinitely in the absence of substrate.

Drug-Fastness. Microorganisms may become adapted to the action of antibacterial substances, and by a process of training acquire the ability to grow in concentrations that are bacteriostatic for the unadapted parent strain. This adaptation is of particular interest in relation to the antibacterial agents of chemotherapeutic importance, including certain of the dyes, the sulfonamide compounds, and the antibiotics, penicillin and streptomycin in particular. Such adaptation occurs readily *in vitro* when the microorganisms are grown in increasing concentrations of the antibacterial agent, and there is convincing

¹⁸ Stephenson and Stuckland: *Biochem. Jour.*, 1933, 27:1528.

¹⁹ Stephenson and Yudkin: *Biochem. Jour.*, 1936, 30:506.

²⁰ See Pollock: *Brit. Jour. Exp. Path.*, 1946, 27:419.

²¹ Hinshelwood: *Chemical Kinetics of the Bacterial Cell*. Clarendon Press, Oxford 1946.

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evidence that it also occurs *in vivo* though the relative importance of the latter phenomenon is not altogether clear. Strains of pathogenic microorganisms that have become resistant to the action of antibacterial substances in concentrations within a chemotherapeutic range are said to be drug-fast.

From a practical point of view it is significant that such adaptation can occur *in vivo*. Chemotherapy of the parasitic infections, such as trypanosomiasis, is of long standing and the acquisition of drug-fastness by such parasites is well known. Similarly, the development of strains of *Treponema pallidum* which are drug-fast to the arsenicals is observed from time to time. The occurrence of drug-fast strains of bacteria has assumed importance with the advent and general use of antibacterial agents effective in the chemotherapy of bacterial infections, first the sulfonamides and later the antibiotics. For example, a striking increase in the proportion of drug-fast gonococci occurred with the general application of sulfonamide therapy of gonorrhea. Carpenter *et al.*²² observed an increase in the incidence of sulfonamide-fast strains of the gonococcus from 15 to 59 per cent in a period of fifteen months; similar results have been reported from Britain, and it is a common observation that at the present time the majority of cases of gonorrhea do not respond to sulfonamide therapy.

The prophylactic use of chemotherapeutic drugs also results in the development of drug-fast strains of bacteria. Thus, Siegal, Karr and Julianelle²³ found that sulfadiazine prophylaxis produced drug-fast pneumococci which spread, infecting some 90 per cent of the group under observation, and the institution of sulfadiazine prophylaxis of streptococcal infection in naval training camps in the United States during World War II was followed by a tremendous proportionate increase in drug-fast strains of streptococci within these groups which produced streptococcal infections that did not respond to sulfonamide therapy (p. 359). Cases of infection with sulfonamide-fast pneumococci have been found and the development of drug-fast strains during therapy and their spread to other persons have been observed; as yet, however, no widespread prevalence of infection with sulfonamide-fast pneumococci has been observed, and according to Hamburger *et al.*²⁴ the occurrence of drug-fast strains has not been quantitatively important in clinical practice as yet.

The widespread use of penicillin has resulted in an increase in penicillin-fast strains of staphylococci; in Hammersmith Hospital in London 14.1 per cent of strains isolated from lesions in the period April–November, 1946, were penicillin-fast, and this rose to 38 per cent for the period February–June, 1947.²⁵ Drug-fastness to streptomycin is especially prone to occur, and develops so rapidly in the infected individual undergoing therapy as to limit somewhat its chemotherapeutic value. It is not clear whether the observed increasing proportion of drug-fast strains of bacteria results from an adaptation to the drug *in vivo* and spread of the fast strain, or whether the drug-

²² Carpenter, Ackerman, Winchester and Whittle: *Amer. Jour. Pub. Health*, 1944, 34:250.

²³ Siegal, Karr and Julianelle: *Amer. Jour. Hyg.*, 1945, 41:228.

²⁴ Hamburger, *et al.*: *Jour. Inf. Dis.*, 1943, 73:12.

²⁵ Barber *Brit. Med. Jour.*, 1947, ii:863.

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fast strains exist prior to therapy and occur in larger proportion through elimination of susceptible strains by chemotherapy. It is not unlikely that both processes are operative, but their relative importance is not known. In any case it is clear that virulent, drug-fast strains of bacteria can and do develop and become disseminated through the host population.

As indicated elsewhere (p. 155), antibacterial agents affect the physiological processes of the cell, and it follows that the nature of the modification that results in drug-fastness is essentially one in the physiology of the bacterium. There is evidence for three general kinds of modification, viz.:

(1) If the drug acts by interference with some particular metabolic process, it is not unreasonable to suppose that the drug-fast strain may by-pass that process and make use of some alternative mechanism. If so it might be expected that the fast strain would differ physiologically from the parent strain. Such physiological differences have, in fact, been observed in sulfa-pyridine-fast pneumococci which, while dehydrogenating glucose at a rate equal to that of the parent strain, cannot dehydrogenate 3-carbon intermediates such as glycerol, lactate and pyruvate, while the parent strain can, and show also a marked diminution in hydrogen peroxide production.²⁶ If, however, the drug interferes with a synthetic process, the modification would not appear in the respiratory metabolism. While the processes of synthesis are much less well understood, there is evidence which suggests that alternative pathways of synthesis may be developed, as in the syntheses involving pantothenic acid catalysis by streptococci²⁷ and pantothenic acid and tryptophane metabolism of staphylococci.²⁸

(2) Since the action of the sulfonamides is antagonized by *p*-aminobenzoic acid, it has been suggested that in some instances sulfonamide-fast strains of bacteria are resistant by virtue of an increased production of this or similar antagonists. In support of this it has been found that sulfonamide-fastness of staphylococci and gonococci is in some cases associated with the production of increased amounts of *p*-aminobenzoic acid,²⁹ and it has also been shown that pantolyltaurine-fast strains of the diphtheria bacillus are resistant because of their ability to synthesize pantothenic acid.²⁷ Specific antagonists of many antibacterial substances, including the antibiotics, are not known and the extent to which this general explanation of drug-fastness can be tested is not great as yet.

(3) A third possible explanation is that, if the drug acts as a competitive inhibitor of an essential metabolite because of similarity in molecular structure, the adaptation may consist in the development of the ability to metabolize the drug instead of the metabolite, giving rise to an apparently anomalous situation in which the essential metabolite shows antibacterial activity. A number of observations in support of this view have been reported. It was found by Wooley³⁰ that strains of the yeast *Endomyces vernalis* which were inhibited by pyriithamine, an analogue of thiamine, could be made resistant

²⁶ MacLeod. *Proc. Soc. Exp. Biol. Med.*, 1939, 41:215.

²⁷ Mellwain. *Brit. Jour. Exp. Path.*, 1943, 24:203.

²⁸ Sevag and Green. *Jour. Bact.*, 1944, 48:615, 623, 631.

²⁹ Landy, Larkum, Oswald and Streightoff. *Science*, 1943, 97:265, Spink. *Jour. Exp. Med.*, 1944, 79:331, Landy and Gerstung. *Jour. Immunol.*, 1945, 51:269.

³⁰ Wooley. *Proc. Soc. Exp. Biol. Med.*, 1944, 55:179.

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by cultivation in its presence, and simultaneously acquired the ability to utilize pyriithiamine instead of thiamine. Emerson and Cushing³¹ reported that a strain of the fungus *Neurospora*, made sulfonamide-fast by cultivation in the presence of sulfanilamide, not only utilized the drug but required it as an essential metabolite, and that the drug-fast strain was inhibited by *p*-aminobenzoic acid, the reverse of the behavior of the parent strain. Similarly, streptomycin-fast variants of meningococci were found by Miller and Bohnhoff,³² some of which required streptomycin for growth, *i.e.*, were streptomycin-dependent, and others were only streptomycin-fast. Streptomycin-fast dependent variants of staphylococci, enteric bacilli, etc., have been described by Kushnik *et al.*³³ and by Paine and Finland.³⁴ The role of streptomycin as an essential metabolite for such variants is also strongly supported by the studies of Rake.³⁵

There are, of course, still other explanations of the basis of adaptive resistance to antibacterial agents. For example, a degree of tolerance might also result from the development within the cell of substances which decrease the solubility of the drug and hence its ability to penetrate the cell; such an explanation has, in fact, been suggested to account for the tolerance which certain fungi may develop for disinfectants. Or the drug-fast variant may inactivate the antibacterial agent by decomposition; naturally occurring penicillin-resistant strains of staphylococci often produce penicillinase, and penicillin-inactivating substance is formed by some penicillin-fast variants.³⁶

It is not necessary to assume that a single mechanism is operative in the development of drug-fastness, *i.e.*, those indicated above are in no sense mutually exclusive, and it is, in fact, probable that a variety are functional. For instance, while sulfonamide-fastness of gonococci is sometimes associated with an increased production of *p*-aminobenzoic acid as noted above, all sulfonamide-fast gonococci do not produce antagonists. Furthermore, more than one mechanism may be operative in a single adaptation; Davies and Hinshelwood³⁷ have reported, for example, that in the adaptation of *Bact. aerogenes* to sulfanilamide there is first a reduction in the prolonged lag period produced by the drug which they interpreted as the development of an alternative growth mechanism, and later an enhanced growth rate due to the formation of an antagonist.

The development of drug-fastness is most commonly a gradual process, though there are exceptions such as the streptomycin-fast dependent meningococci which appear on initial culture. The bacteria are usually cultured in increasing concentrations of the drug over an extended series of transplants and become more and more drug-fast in that successively higher concentrations are tolerated. These sometimes become almost fantastic in the light of the original sensitivity. The drug-fastness is specific also in that a sulfonamide-fast strain is normally susceptible to penicillin, etc. This specificity may

³¹ Emerson and Cushing: Jour. Bact., 1947, 54:195.

³² Miller and Bohnhoff: Jour. Bact., 1947, 54:467.

³³ Kushnik, Randles, Gray and Birkeland. Science, 1947, 106:587.

³⁴ Paine and Finland. Science, 1948, 107:143.

³⁵ Rake. Proc. Soc. Exp. Biol. Med., 1948, 67:249

³⁶ See North and Christie: Med. Jour. Australia, 1946, 33:176.

³⁷ Davies and Hinshelwood. Trans. Faraday Soc., 1943, 39:431.

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extend within a group of closely related compounds such as the sulfonamides, and Harris and Kohn³⁸ have reported that acquired resistance of *Bact. coli* to sulfanilamide did not always parallel that to sulfathiazole. Or it may extend to other compounds; thus Hinshelwood²¹ has shown that *Bact. aerogenes* adapted to proflavine shows some "cross adaptation" to other acridines, methylene blue, and propamidine. In general, the specificity is greatest in the early phases of the adaptation. As indicated above, drug-fastness may be temporary in that reversion occurs on continued culture in the absence of the drug, but when the adaptation is carried over many transplants and to relatively high concentrations of the drug, it is apparently permanent. Reversion may be induced in some instances, however, by adaptation to some other drug, and acriflavine-fast *Bact. aerogenes* becomes susceptible to acriflavine when adapted to phenol.

Immunological Variation. The loss of capsules in the conversion of S to R forms is reflected, not only in the morphology and virulence of the organism, but also in its immunological character. The capsular material, usually polysaccharide in nature, confers a type specificity upon the organism, while the cell body proper contains antigens, designated as somatic, which confer an immunological relation upon closely related forms having similar or identical somatic antigens but different type-specific antigens. The change from S to R is, then, ordinarily accompanied by a loss of immunological specificity. The pneumococcus types, for example, differ from one another by virtue of type-specific capsular material and in the rough form lose their type specificity and become immunologically identical (p. 385).

Another type of colony variation or dissociation connected with the presence or absence of flagella was originally observed in cultures of *Proteus vulgaris*. The ordinary type of *Proteus* colony is irregular and tends to spread in a thin film over the surface of an agar medium because of the pronounced motility of these organisms, but occasionally compact, discrete colonies are observed. The spreading type has been designated by the letter H (German *Hauch* = film) and the discrete form by the letter O (German *ohne Hauch* = without film). The distinction between H and O forms has come to have considerable significance because of the difference in the antigens present in the flagella and those present in the cell body, the H or flagellar antigens conferring, like the capsular polysaccharide of some organisms, a type specificity upon the bacterium. The O antigens, though often referred to as somatic antigens, are by no means strictly analogous to the somatic antigens of the rough forms of bacteria, as will appear.

The nature of the H-O variation would appear to be different from that of the S-R variation in that the former takes place somewhat more readily and does not have a semipermanent character. The formation of flagellar antigen, for example, may be suppressed by cultivation of the bacteria on phenol agar, a suppression which is immediate but not permanent, for transfer to nutrient agar results in the prompt reappearance of the H antigens. Furthermore, the H antigens of *Salmonella* have been found to be of two kinds, specific and non specific (the non-specific having even broader group affiliations than the O antigens). Bacterial species having both specific and

³⁸ Harris and Kohn. Jour. Immunol., 1943, 46: 169.

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the cyclical processes of carbohydrate metabolism like the alcoholic fermentation, the Krebs cycle, etc. The individual reactions are determined by the concentration of intermediates, the amount of enzyme and the reaction velocity. A steady state occurs during logarithmic growth, but with the exhaustion of food materials, accumulation of metabolic products and the like, there is cessation of the maximum growth rate and concentrations of diffusible intermediates fall, and the individual enzymes decay and alter in their relative proportions as the system moves toward a new equilibrium. On transfer to fresh medium readjustment occurs.

toward an equilibrium consistent with maximum growth rate. Thus, the relative proportions of the elements of the catalytic system and the concentrations of substrates of the individual reactions exist in a series of metastable states of enzyme balance under ordinary culture conditions. For example, the formation of bacterial deaminases is suppressed during rapid growth in the presence of fermentable carbohydrate and does not occur at acid reactions but decarboxylases are formed at an acid reaction, and both kinds of enzymes are formed in the later stages of culture growth; the kind and proportion of enzymes present is a function of the age and condition of the culture. Similarly, the products of carbohydrate metabolism depend upon environmental factors, again a matter of balance in the function of the cellular enzymes.

If a strain of bacteria is transplanted to a medium differing from the one in which it has been grown, a greater degree of adjustment occurs. For instance, *Bact. aerogenes* ferments both glucose and lactose, but if cultured continuously on glucose and then transferred to lactose, the growth rate, measured as the mean generation time, is reduced. After several transfers in lactose the rate increases and the strain grows as rapidly in lactose as it formerly did in sucrose. If the training to lactose is only partial, reversion occurs in non-lactose-containing media, but if the strain has been carried through many transplants in lactose, the ability to use the sugar with a maximum efficiency persists. Since the bacterium ferments both sugars with facility the adaptation is not overt, but it is, nevertheless, a typical adaptation, and the result is clearly a consequence of alteration in the balance of enzymes already existing in the cell. The adaptive process is much more striking, and less obviously a result of change in enzyme balance, when there is a great differential between the parent and adapted strains, as between no detectable acidity and an active fermentation, whether it occurs slowly on successive transfer or very rapidly as in the formation of an adaptive enzyme.

The change in enzyme balance may be quantitative in nature. The enzyme in question may be present only as a precursor, the formation of enzyme occurring in the presence of substrate through mass action as suggested by Yudkin⁴⁹ for adaptive enzyme formation. Or the enzyme may be initially present in only very small amount, on the basis of comparison between number of molecules of certain vitamins per cell and the turnover rate of several enzymes, McIlwain⁵⁰ indicates how some enzymes may be present as only one or a few molecules. Or, finally, catalysis of the new reaction may be a function of an

⁴⁹ Yudkin Biol. Rev., 1938, 13:93.

⁵⁰ McIlwain Nature, 1946, 158:898.

existing enzyme but at a relatively low reaction velocity, and adaptation a matter of expansion of that enzyme. The last opens the question of qualitative modification of the enzyme, in that distortion of its specificity by a slightly different substrate requires greater activation energy and hence a lowered reaction rate, but if the substrate is present during formation of the enzyme, and the distortion not too great, a modified pattern may eventually result. This is consistent with much of the observed data but there is no direct evidence regarding it.

Drug-fastness is also satisfactorily explained in terms of enzyme balance. As indicated earlier, drug-fastness may result from the production of an inhibitor such as *p*-aminobenzoic acid in excess amounts, a qualitative modification such that the drug displaces the antagonist as an essential metabolite, or by diversion to an alternate metabolic pathway. The first of these is clearly an expansion of an existing catalytic system. Regarding the second, suppose it be assumed that the drug displaces the prosthetic group of an enzyme, *viz.*, panto-lyltaurine, to give a modified enzyme and the formation of different metabolic products. The adaptation can thus consist of an altered enzyme balance to allow the metabolism of the new intermediates, and when this is established, the original essential metabolite competitively inhibits the new system and the drug has become an essential metabolite. The development of an alternate metabolic pathway is likewise an expansion of an existing enzyme or system of enzymes which ordinarily contributes in but small amount to the maintenance of the concentration of a given intermediate, perhaps because of lower reaction velocity. When the function of the usual system is inhibited by the drug, the alternate is expanded, perhaps only quantitatively, to neutralize the effects of the lower reaction velocity. The whole is analogous to an industrial system in which a raw material for a given process is shut off, an alternative, more expensive process is developed and the economies effected in large scale operation make it as efficient as the original process.

THE INTERPRETATION OF THE PHENOMENA OF VARIATION

The significance of the phenomena of bacterial variation to biology is uncertain. Because of unique environmental factors interpretation of the morphological variation of individual cells presents unusual difficulties, and neither the mechanisms underlying this type of variability nor its relation to variation in anatomical structures of higher organisms is apparent as yet. Variation in colonial morphology, however, appears to be associated, in part, with physiological and immunological variation, and it is not unlikely that changes in colony form arise as a consequence of alterations in the nature of the cell surface.

The significance of the apparent loss and gain of physiological characters, even if these be absolute in that a property is completely lost or absent, is difficult to assess in general biological terms. As Needham²¹ has pointed out, although a structure which makes possible a given physiological function may not be regained after being lost, the organism may re-acquire the function through the development of another and different structural mechanism. It is clearly open to question as to whether an enzyme system lost or gained by a

²¹ Needham: *Contributions of Chemical Physiology to the Problem of Reversibility in Evolution*. *Biol. Revs.*, 1938, 13:225.

bacterium may be regarded in the same category as an anatomical structure such as a tooth.

The immunological characters of bacteria, however, have their counterparts among the higher organisms in which immunological characters are known to be inherited, as in the case of human blood groups, or parallel phylogenetic relationships of accepted zoological classifications, as in the immunological relationship of blood proteins. In this connection it has been shown⁵² that the immunological character of serum proteins of pigeons is genetically determined. If immunological characters of bacteria are to be regarded as fundamentally significant, it appears that on the one hand bacteria are unstable in this respect, *viz.*, the constant fluctuation of specific and non-specific flagellar antigen in the diphasic *Salmonella*, and on the other, that such characters may be acquired through environmental influence. The fluctuating variation between specific and non-specific H antigens and the random redistribution of these antigens in the bacterial population following selection may well indicate, however, that some, at least, of these immunological characters may not be of fundamental biological importance, a suggestion that is, in part, supported by present knowledge of common antigens and partial antigens.

This apparent acquisition of new immunological characters is also of interest in a somewhat different connection, that of the apparent autocatalytic properties of some substances. Recent studies have indicated that some of the filterable viruses are proteins. Since these agents are able to increase in the body of the host, it would appear that they stimulate the host cells to form more virus protein. The analogy to the transmutation of pneumococcus types and the acquiring of the heterophile antigen by the typhoid and paratyphoid A bacilli is obvious.

Bacterial Phylogeny. From the evidence of comparative bacterial physiology (Chapter 4) it appears not unlikely that bacteria were among the first living organisms on the earth. The autotrophic bacteria may plausibly be regarded as primitive forms of life that could exist in an environment containing no organic matter, and that have persisted until the present time. Teleologically, it would seem that in the bacteria nature tried a variety of energy-yielding mechanisms ranging from the oxidation of inorganic compounds of nitrogen, carbon, sulfur, iron, manganese, etc., to the utilization of radiant energy by the photosynthetic forms. The last process, however, became of quantitative importance in the green plants rather than in the bacteria. With the accumulation of organic matter, transitional forms, persisting today as the facultative autotrophes, arose in which there appeared mechanisms for the oxidation of organic compounds, concurrent with this development came the ability to respire in the absence of molecular oxygen. The wide variety of bacteria which exist at the present time may well be regarded as a consequence of the physiological expansion made possible by the accumulation of a variety of organic compounds and the development of respiratory mechanisms that made possible their utilization. In this connection it is of interest that vestiges of autotrophic physiology, such as the ability to oxidize hydrogen, persist in some of the heterotrophic bacteria.

With this development of respiratory mechanisms, however, a series of de-

⁵² Cumley, Irwin and Cole *Proc. Nat. Acad. Sci.*, 1941, 27:565.

Hirst (1941) protected mice infected with Group C, but not those with Group A, by treatment with leech-extract hyaluronidase. Blundell (1942) found only a feeble protective action in bull-testicle hyaluronidase in Group A infections; and McClean (1942) none at all, in either Group A or C infections, with doses of enzyme in excess of those required for decapsulation of the streptococci *in vitro*. Kass and Seastone (1944), however, report that bull-testicle hyaluronidase increases the bactericidal power of normal blood for Group A streptococci, and will protect mice against Group A infection. (See also Rothbard 1948.)

It is possible that the Vi antigen of *Salm. typhi* (see Chapter 30), which is associated with the virulence of the organism, may act analogously to the capsular substances of the cocci. It is only one-fiftieth as toxic as the O endotoxin (Henderson and Morgan 1938); nevertheless, intravenous injection of extracts of Vi bacilli greatly reduces the protective effect of Vi antisera in mice infected with the living bacilli (Henderson 1939; see also Leon and Morales 1943). In animals with protective O antibodies, it is possible that the Vi antigen is an effective aggressin if it covers the surface of the infecting bacterium, thereby masking the O antigen and preventing opsonization by the O antibody (see Felix and Pitt 1951).

The "complete" antigens of enterobacteria, as extracted by the trichloroacetic acid method of Boivin, are both specifically and non-specifically aggressive (Boivin and Delaunay 1942, 1943, d'Alessandro 1951-52). The non-toxic polysaccharide hapten component of this lipo-protein-polysaccharide complex is also specifically aggressive, presumably because it neutralizes opsonic antibody (Boivin and Delaunay 1945). The non-specific effect of, for example, a *Bact. coli* complete antigen on a *Salm. typhi-murium* infection of mice, appears to be a consequence of intoxication of the experimental animal.

Aggressins of this kind are not confined to enterobacteria. Complexes isolated by the trichloroacetic acid method from virulent human tubercle bacilli will enhance the infectivity of tubercle bacilli for guinea-pigs, whereas similar extracts of attenuated strains do not (Raffell 1947).

The neutralization of protective antibody by soluble antigens is unlikely to have any general effect on infections of the non-immunized animal until antibodies are formed in response to the infection; and, as we noted in the last chapter, serum antibodies do not usually appear until the second or third day (see, e.g., Wood *et al.* 1946). Another action of antigens is possible, which may affect an earlier stage of the disease although we have no evidence as yet that it occurs, namely, the paralysis of antibody formation, either local or general, by overwhelming doses of antigen (see Felton 1949 and Chapter 50).

In addition to these dissolved haptens or antigens it is clear that some infective exudates will contain other more or less toxic substances of bacterial origin that interfere with the defence mechanism of the host, as we have already noted in anthrax infection. Both Pettersson (1940) and Stevenson and Reed (1940) record a negative chemiotactic effect in *Staph. aureus*; the substances responsible appeared to be distinct from the recognized components of staphylococcal toxin. Pettersson's substance, which he calls "negatactic," was antigenic, and antisera prepared against it had a protective effect in guinea-pigs and mice. Pettersson (1943) also described negatactic substances, distinct from capsular substances, in *B. anthracis* and pneumococci. The anthrax anti-negatactic sera appeared to act by permitting the infiltration into the infected tissue of active phagocytes. The pneumococcal negatactic substances displayed some degree of host-specificity; the phagocytes of man and of the rabbit were susceptible to its action, but those of the mouse and guinea-pig were not.

The frank toxins of a bacterium may have an undoubted specific action on the mesenchymatous defence cells of the host. Two pathogenic species, at least, *Staph. aureus* and *Str. pyogenes*, produce a specific leucocidin that attacks the polymorphonuclear cells, and, for example, Dennis and Senekjian (1939) report an antigenic leucocidin in *Salm typhi*. We know little as yet about the action of

generative changes appeared, the first being the loss of the ability to reduce carbon dioxide, followed by an increasing dependence upon preformed organic compounds as building stones for protoplasm and upon preformed components of the enzyme systems concerned in respiration, such as coenzyme, thiamine and the like. Degeneration appears most marked in the pathogenic forms (possibly reaching an ultimate in the filterable viruses if, as some think, these are microorganisms completely dependent upon the host cells for enzyme systems), and it is possible that this degeneration has been accelerated through parasitism.

Such an approach necessitates the assumption that the autotrophes appeared fully developed and in very many respects this seems highly improbable. An alternative which avoids this difficulty is the assumption that the formation of organic matter, through the condensation of small polyfunctional molecules with aggregation into macromolecules, preceded the appearance of living cells, and that these were essentially heterotrophic. From such heterotrophic ancestors the autotrophic bacteria could develop as the supply of preformed organic matter ran low, and present heterotrophic forms utilizing organic matter of green plant origin differentiated either from or coincidentally with the autotrophes.

Parasitism.⁵¹ Although the ability of some bacteria to produce disease may be a purely fortuitous coincidence, viz., the tetanus bacillus, many of the pathogenic forms have, through long association, become adapted to life in the body of the host to such a degree that they are unable to survive in nature or possibly even on artificial culture media or in the bodies of animals closely related to the particular host. This seems, for example, to be the case with the leprosy bacillus, which, so far as is known, is not able to grow anywhere except in the body of man and possibly of the anthropoid ape. Theobald Smith has suggested that bacteria of great pathogenic power should be regarded as incompletely adapted parasites that have not yet succeeded in establishing an equilibrium between themselves and their host. The less complete the adaptation, the more virulent the disease produced. This concept would explain the tendency of long established diseases to decrease in severity at the same time that they are becoming more prevalent.

This adaptation to a parasitic mode of existence is indicated in a variety of ways. The nutritive requirements of some of the pathogenic bacteria, for example, such as the necessity for fresh blood, ascitic fluid and the like in laboratory media, suggest an adaptation to an environment in which these or similar substances are available—the tissues of the host. The optimum temperatures for the pathogenic bacteria are, without exception, the body temperatures of their host, the human type of tubercle bacillus grows best at 37° C. but the avian type is presumably adapted to the higher body temperature of the bird, 41° to 42° C.

The question of whether a host may, through long association, become adapted to a parasitic bacterium to such a degree that the presence of the microorganism is either necessary or of advantage to the continued existence of the host is an open one. In certain cases the presence of a parasitic bacterium

⁵¹ Cf. Smith, Theobald *Parasitism and Disease*, Princeton University Press, Princeton, 1934.

There is, perhaps, something to be said for the term *aggressive action*, if we can so define it as to make it fill a gap in our terminology. It would seem to be most usefully employed as indicating an action on the cells or body fluids that is not directly cytotoxic, or of which the directly injurious effects are minimal, but which so interferes with the defence mechanism of the host as to favour, specifically or non-specifically, the multiplication of bacteria within the tissues.

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bears a significant relation to the assimilation of food by the host; such, for example, is the case with respect to the leguminous plants and the root nodule bacteria, and the herbivorous animals and cellulose-decomposing microorganisms. Whether the abundant intestinal flora of man and other animals in general functions in a similar fashion has been the theme of considerable speculation and some investigation. Nuttall and Thierfelder early showed that guinea pigs removed by cesarean section and kept in a sterile environment survived for ten days or so, but Schottelius was not successful in rearing bacteriologically sterile chicks. Nutritional inadequacies of the chick diet apparently accounted for these results, for it was later shown that chicks could be raised for as long as forty days in a bacteria-free environment. The work of Reyniers⁵⁴ and his colleagues in recent years has shown that not only chickens, but a variety of higher animals such as guinea pigs, rabbits, rats, etc., may develop in the complete absence of bacteria. It appears likely, therefore, that an intestinal flora is not essential to the continued existence of some of the higher animals, but whether it may be an advantage is as yet uncertain.

⁵⁴ Reyniers: *Microsurgical and Germ-Free Techniques: Their Application to Experimental Biology and Medicine*. Charles C Thomas, Springfield, Ill. 1943.

of cases, a concentration over 1/100 to 1/20 unit per ml. of blood ensures a negative Schick reaction.

The proportion of persons with diphtheria antitoxin in the circulating blood varies in a striking and characteristic way as we pass from birth through childhood and adolescence to maturity and old age. Table 75 sets out the extensive figures recorded by Zingher (1923), where the percentage of positive reactors is tabulated.

At about 6 months of age just over half the infants react positively. The percentage of positive reactors then rises rapidly and between 8 months and 3 years fluctuates about an average of 88 per cent. After this, there is a continuous fall, at first rapid, then more gradual, till at the allotted span of three-score years and ten the proportion has dropped to approximately 5 per cent.

TABLE 75

COMPOSITE TABLE SHOWING PERCENTAGE OF SCHICK-POSITIVE REACTORS IN VARIOUS AGE GROUPS IN NEW YORK CITY AND NEW JERSEY.

Age Group.	Number Tested.	Per cent. Positive.
6-7 months	53	56.6
7-8 "	41	63.4
8-9 "	62	83.8
9-10 "	58	93.1
10-11 "	61	87.0
11-12 "	34	91.1
1-3 years	1,727	83.2
4-6 "	1,328	58.6
6-7 "	13,754	50.4
7-8 "	16,180	43.5
8-9 "	17,126	36.6
9-10 "	18,065	32.2
10-11 "	18,057	29.3
11-12 "	17,994	28.2
12-13 "	16,258	26.6
13-14 "	14,138	23.1
14-15 "	9,650	19.7
15-16 "	4,861	17.8
16-17 "	369	18.4
20-30 "	1,253	11.7
30-40 "	1,488	10.6
40-50 "	1,220	8.2
50-60 "	920	6.4
60-70 "	662	5.4
Over 70 "	181	5.5

The Origin of the Natural Antitoxins.

The most generally accepted view of the immunological events underlying this observed fluctuation in skin-sensitivity to diphtheria toxin is as follows. Most new-born infants have circulating antitoxin acquired passively from their mothers—mainly by passage to the foetus during pregnancy, in part perhaps with the colostrum in the early days of suckling. This passive congenital immunity, like all other types depending on the passive acquirement of antibodies, will be relatively short-lived. A careful study of the disappearance of antitoxin from the blood of an infant whose mother was highly immune is recorded by Neill and his colleagues (1932). From infancy onwards the child will be exposed to the risk of infection, increasing from year to year during the earlier part of school life. Some children

THE CLASSIFICATION OF BACTERIA

The classification of bacteria presents peculiar difficulties that stem more or less directly from their simplicity of structure. Since taxonomy in general rests on a morphological or anatomical basis, the relationship, phylogenetic or otherwise, of bacteria to the higher forms is difficult to define, and the interrelations of the bacteria themselves present problems to which taxonomy has, to date, offered no entirely satisfactory solution. A consequence of this structural simplicity is the widespread use of physiological characters in the differentiation of the bacteria into genera and species, characters whose counterparts among the higher organisms are, in general, regarded as of but minor taxonomic importance. As has been pointed out previously, differences that are quite possibly trivial biologically are often of great practical importance and hence have come to assume a taxonomic significance that may often be entirely undeserved. In other cases the biological importance of a given characteristic or group of characteristics is unknown, and the inability to assess the significance of characters proposed as differentials has led to disagreement among bacteriologists.

Such a situation clearly does not lend itself to order and system nor, and perhaps most important of all, does it allow the development of a classification which is based upon characters of fundamental significance and which shows the biological or genetic relationship of these organisms to one another. The practical consequences are two—there is no classification of bacteria which is generally accepted in more than its primary subdivisions, and, second, the bases upon which various groups of bacteria are classified differ widely, as will appear, from one group of organisms to another. It should be emphasized, therefore, that bacterial "species" are by no means analogous to the species of the zoologist or botanist and cannot be compared directly to them.

The phenomenon of bacterial variation, discussed in the previous chapter, is clearly of no small importance in the classification of these organisms. From the general biological point of view there is but little solid ground upon which a sound taxonomic structure can be built. In the practical identification of bacteria, however, such variations are not a source of great embarrassment. As has been pointed out, the majority of bacterial variations arise as a consequence of alterations in the environment and, in a working sense, may be regarded as adaptations. The observed uniformity of the morphology and biochemical properties of the bacterial culture is due to the fact that observations of these characters are always made in the same way. It is only when the procedure is varied, either in terms of the past history of the bacterial culture or in the

antibodies in the maternal blood must pass in order to reach the foetal circulation ; or that the route was necessarily *via* the placental vessels. Hartley's (1948) experiments with pregnant guinea-pigs passively immunized by diphtheria antitoxins prepared in different animals and refined by different methods indicate that uterine transmission is selective, homologous guinea-pig antitoxin passes to the foetus far more readily than the heterologous horse antitoxin, and salt-precipitated antibody globulins pass more readily than those altered and refined by peptic digestion (see Chapter 7).

Brambell and his colleagues (1948, 1949, 1950, 1951, 1952, 1953) record a similar selectivity in the pregnant rabbit, where the passage of horse and bovine agglutinin and antitoxin is much less than that of the corresponding antibodies prepared in the rabbit. In this animal they also found little evidence of placental transmission. After the establishment of the allanto-chorionic placenta, in the 15-day embryo, maternal antibodies reached the foetus *via* the uterine cavity, passing through the endoderm of the yolk sac splanchnopleure into the vitelline circulation, whose integrity was proved to be essential by the interruption of transmission when the vitelline vessels were ligated. In man, however the allanto-chorionic placenta appears to be the probable route of uterine transmission. They regard the vitelline route in the rabbit as analogous to adsorption of colostral antibody from the gut of new-born animals, since the yolk sac is morphologically an extension of the gut. They also record that antibody in the amniotic fluid, though it accumulates in the foetal stomach, does not reach the foetal circulation by this route.

TABLE 76

THE RELATIVE IMPORTANCE OF THE UTERINE AND MAMMARY ROUTES IN THE PASSIVE TRANSMISSION OF ANTIBODIES FROM MOTHER TO OFFSPRING. (After Mason, Dalling and Gordon.)

Species	Layers of Tissue between Maternal and Foetal Circulation	Importance of Uterine Transmission	Importance of Transmission by Colostrum.
Pig	5	—	+++
Ruminants	4	—	+++
Carnivores	2	±	+
Rodents, apes, man .	1	+++	±

In man, antibody is present in the colostrum, though usually in small amounts, unless the blood antibody levels are high. Immediately after delivery, for example, the diphtheria antitoxin content of the colostrum may be as high as one-third that in the blood, but it drops steeply during the first days of lactation. Some transmission *via* the colostrum appears to take place (Sugg 1935, Magara 1936); though Schubert and Grunberg (1949), who recorded in human colostrum significantly higher typhoid agglutinins than in the blood, found that none of this antibody was absorbed by the babies at breast. In sheep immunized with diphtheria toxoid, mean concentrations in the colostrum at parturition as high as 7.8 times those in the serum are recorded, the concentration, however, drops rapidly within a day to that in the serum, as a result of suckling the lambs (Barr *et al.* 1953). Mice differ from other rodents in the table in that the mammary route appears in some cases to be more important than the placental. Thus, a higher degree of milk transmission of antibodies was demonstrated experimentally in mice infected with herpes virus (Berry and Slavin 1943) and with *Trypanosoma duttoni* (Culbertson 1940); and, in mice infected with Coxsackie virus, the passive transfer of maternal antibody appears to be solely colostral (Melnick *et al.* 1950). In mares with high-titre tetanus antitoxin, Lemétayer and his colleagues (1947) found some uterine transmission, though mammary transmission predominated.

way in which the test is conducted, that variability becomes apparent and the illusory nature of the supposed constancy of these organisms is demonstrated.

The question of whether bacteria should be regarded as members of the plant or animal kingdoms is no longer considered of great importance. Possessing characteristics of both, these organisms may be regarded as a connecting link between the plants and animals. Using the term bacteria in its broad sense, the microorganisms included under this head make up a continuous series of types which appear to connect the two kingdoms. The so-called higher bacteria, the sheathed, filamentous forms, are closely akin to the fungi, bacteria such as the tubercle bacillus and the diphtheria bacillus appear to be further removed from the plants but show some fungoid characters, such as tendencies to branching and filament formation. Among the well-known forms such as the streptococci, staphylococci, sporulating rods and gram-negative intestinal forms, the morphological relations to the fungi are less apparent, organs of locomotion appear, and with the spiral forms the bacteria merge into the protozoa. Physiological characters and the evidence of chemical composition of the cells tend to reinforce this intermediate position. The metabolism of these organisms is chemosynthetic, an animal-like character, but the ability to utilize ammonium salts as a source of nitrogen suggests a plant type of physiology, nucleic acids found only in plant cells or only in animal cells have all been found in the bacteria. It was proposed many years ago that a third kingdom be created for these organisms, the *Protista*, but since the dividing lines between such a third group and the plants on the one hand and the animals on the other are as vague as that between the plants and animals, the proposed new kingdom would solve no difficulties and has never been accepted. It is generally agreed, however, that the sum total of bacterial characters allies them more closely to the plants than to the animals, and they are classified with the plants as *Schizomycetes* or fission fungi (German *Spaltpilzen*). The relationship of the *Schizomycetes* to other plants may be indicated as follows:

- Phylum I. *Thallophyta*, plants without distinction of root, stem and branch
 - Subphylum 1—the algae
 - Subphylum 2—the fungi—thallophytes lacking chlorophyll
 - Class I. *Schizomycetes*—the bacteria
 - Class II. *Myxomycetes*—the slime molds
 - Class III. *Phycomycetes*—the algae-like fungi
 - Class IV. *Ascomycetes*—the fungi forming ascospores
 - Class V. *Basidiomycetes*—the fungi forming basidiospores
- Phylum II. *Bryophyta*—the mosses
- Phylum III. *Pteridophyta*—the ferns
- Phylum IV. *Spermatophyta*—the seed-bearing plants

Although the interrelationships of the organisms included under *Schizomycetes* present problems which are, in many essentials, new to the taxonomist, certain "natural" groups are apparent. Of these the most obvious is that based on morphology, with primary division into spherical, rod-shaped, spiral and filamentous forms, and subdivision on the basis of spore formation, presence and location of flagella, and staining reactions to the gram and acid-fast stains. The earlier classifications, the better known of which are those of Migula and of Lehmann and Neumann, were made on this basis. These classifications

age is entirely due to natural active immunization resulting from the reception from without of specific stimuli in the shape of clinical or subclinical infections? This hypothesis has certainly not been placed beyond dispute. It has indeed been vigorously disputed, particularly by Hirszfeld (1926) and his colleagues.

Hirszfeld believes that all the normal antibodies should be regarded as "biochemical organs," with which the individual is endowed as the result of his evolutionary history. These "biochemical organs" ripen, or come to maturity, at different periods in his own individual development; just as the various organs and tissues of the body come to full maturity at different ages. As we speak of a "morphogenesis" through which each individual passes, so we should think of a "serogenesis" which forms an integral part of normal development. The time at which any particular antibody normally makes its appearance may be regarded as the period of "immunological crisis" for that particular antibody, and marks the establishment of a "biochemical reflex" on which the production of that antibody depends. Until the biochemical reflex has become established attempts at immunization with the particular antigen concerned will have little effect. Hirszfeld does not deny that specific, or non-specific, stimuli from without may play some part in accelerating the production of particular antibodies, or in increasing their concentration; but he would relegate such influences to a very secondary place. He would not regard the presence in the blood stream of antibodies acting on a particular bacterium as valid evidence of past or present infection with the bacterium in question. Above all, he would not accept the view that the antibody-forming apparatus is a *tabula rasa*, on which environmental stimuli can write at will. It is a complex mechanism with its possible activities fixed and ordered by the phylogeny of each animal species; and it will therefore respond to the appropriate stimuli and to no others.

It will be simpler to discuss the more general aspects of Hirszfeld's views when we have dealt with the origin of antibacterial sensitizers, and to confine our immediate attention to antitoxic immunity, noting in passing that the problem is in no sense a purely academic one but has important bearings on many problems of diagnosis and prevention.

Perhaps the most significant data, supporting Hirszfeld's contention, are to be found in observations (Hirszfeld, Hirszfeld and Brokman 1924, Hirszfeld and Hirszfeld 1927) which suggest a linkage between the inheritance of the power to produce antitoxin and the power to produce the iso-agglutinins which determine the human blood-groups (see p. 1236). From these records Hirszfeld draws the following conclusions. When both parents are Schick-negative the children will be Schick-negative, except during the first few years of life. When both parents are Schick-positive the children will be Schick-positive, and will remain so throughout life. When one parent is Schick-negative and the other Schick-positive the children who belong to the same blood-group as the Schick-negative parent tend to be Schick-negative, those who belong to the same blood-group as the Schick-positive parent tend to be Schick-positive (see also Tamaki *et al.* 1936). The validity of these observations has, however, been questioned by Rosling (1928), who examined 50 children over one year of age whose parents belonged to different blood-groups and reacted differently to the Schick test. He found that 23 children gave the same Schick reaction as the parent to whose blood-group they belonged, while 27 reacted differently.

A negative is notoriously difficult to prove; and we cannot assert that no child has ever developed diphtheria antitoxin except in response to the specific stimulus provided by diphtheria toxin. We can, however, study the frequency of negative and positive Schick reactors in different samples of the population, and correlate our findings with the history of exposure to infection, so far as such a history is obtainable. If we find that free or frequent exposure to infection is consistently associated with a high percentage of negative reactors, and that population groups relatively free from any risk of infection show a notably low percentage of negative

exerted a strong influence on bacterial taxonomy and resulted in the naming of a great many species of bacteria, many of which persist in current classifications.

Morphology is, however, not a sufficient basis for the separation of bacterial species, for many morphologically similar organisms may be quite different in other respects. Physiological differences, generally readily determinable in the laboratory, have been widely used. In fact, one of the early classifications, that of Orla-Jensen, depended upon nutritional differences for the primary subdivision into three main groups:

1. The autotrophic bacteria which, like the green plants, require neither organic carbon nor organic nitrogen compounds
2. Bacteria which require organic carbon compounds but can dispense with organic nitrogen, using ammonium salts or other inorganic compounds of nitrogen
3. Bacteria which, like the higher animals, require both organic carbon and organic nitrogen compounds

A number of workers, especially Kluyver and van Niel,¹ are of the opinion that comparative physiology should constitute the primary basis of separation, rather than being subordinate to morphology.

Beyond such primary subdivisions, classification becomes increasingly difficult and it is quite clear that this is so because not nearly enough is known of the phylogenetic relationships of the bacteria to one another for a detailed classification with definition of genera and species. This question has been discussed in some detail by van Niel² who shows that as yet any classification can be little more than a key and species only "form" species, that is to say, no more than convenient handles.

Bacterial classification has evolved through some sixty odd schemes to perhaps half a dozen current today.³ The criteria made use of are, in the order of approximate fineness of distinction made:

- (1) morphology—both gross and microscopic,
- (2) physiology—biochemical properties,
- (3) pathogenicity—of the disease producing bacteria,
- (4) immunology—the antigenic structure of the bacterial cell.

Of the current classifications that of the Bergey Manual⁴ is by far the most detailed and is the only one that need be considered here. It developed from the earlier classification of Chester and the work of a committee of the Society of American Bacteriologists, but is not, as is sometimes supposed, the official expression of the views of the Society. The Bergey classification has gained ascendancy in this country in recent years, though it is seldom used outside the United States.

An abridged outline of the Bergey (1948) classification is given in the accompanying diagrammatic form. It differs from the Bergey system in that the genus *Staphylococcus* is retained, and the tribe Hemophilae is not broken

¹ Kluyver and van Niel: *Centralbl. f. Bakt., Abt. II*, 1936, 94-369.

² van Niel: *Symp. Quant. Biol.*, 1946, 11-285.

12 years of age. Antitoxin was demonstrated in some of the negative reactors. Yet diphtheria is said to be unknown among the Eskimos.

Kleine and Kroó (1930) tested 101 East African natives by the Schick reaction; 95 of these were children between the ages of 6 and 15, the remaining 6 were adults. In no case was a positive reaction obtained. The toxin employed was subsequently retested and found to have lost none of its activity. Specimens of serum were obtained from 11 of the natives and tested for antitoxin; 10 of these contained antitoxin in considerable amount (0.05 A.U. to more than 1 A.U. per ml.). It is clear therefore that the East African native frequently forms diphtheria antitoxin early in life; yet, according to Kleine and Kroó, this population is not, so far as is known, exposed to infection with diphtheria. (See also Parr and Avery 1926-27, Parr, Goodale and Kirschner 1930.)

Grasset and his colleagues (1933) record a high frequency of natural diphtheria immunity among the Bantu of South Africa, as judged both by Schick tests and antitoxin titrations. They note, however, the occurrence of diphtheria among the populations concerned, and adopt without question the view that the antitoxin has been produced in response to infection. Murray (1943) provides more definite evidence. Among the Johannesburg Bantu clinical diphtheria was rare, but, as Grasset found in his series, the Schick-positive rate was low. In rural school children it was 8.0 per cent. and in urban children 13.7 per cent. The corresponding carrier rates of virulent diphtheria bacilli were 1.8 per cent. in the urban, and 3.2 per cent. in the rural; and though the Schick-positive and the carrier rates did not vary together, immunization was associated with the presence of carriers among the children. It is noteworthy that there was little clinical diphtheria among these Bantu children, as compared with white children in the same district, among whom a similar carrier rate was associated only with an epidemic of diphtheria; no bacilli were found in their throats in non-epidemic periods. Murray (1942) concludes that environmental conditions were in part responsible for the high level of antitoxic immunity of the Bantu, and that the absence of clinical diphtheria in spite of the relatively high carrier rate was due to an ability, perhaps racially determined, to produce antitoxin quickly in response to the specific antigenic stimulus.

In only a few other instances do such surveys include a record of the carrier rate of diphtheria bacilli, or of hæmolytic streptococci, among the population concerned. Wells (1933), records a study of the Schick reactions of central and polar Eskimos, in which a proportion of tests were confirmed by antitoxin titrations, and throat swabs were obtained from all persons examined. He reports the frequent presence of bacilli morphologically resembling *C. diphtheriæ*, and states that four strains gave the typical fermentation reactions, and one strain showed some degree of virulence. These findings clearly tend to throw doubt on the significance of the observations recorded by Heinbecker and Irvine-Jones (1928), but it can hardly be said that the presence of toxigenic diphtheria bacilli was established with certainty.

A study by Asbelew and Margo (1932) of the Schick and Dick reactions of 103 of the 250 inhabitants of the Arctic island of Kolgijew is very difficult to reconcile with the *Durchseuchung* hypothesis—the view that antitoxin is produced only or mainly as the result of infection. With the exception of one girl of 8 years all persons examined were Schick-negative, and with the exception of one doubtful case all were Dick-negative. The antitoxin content of the blood was unfortunately not determined. Diphtheria and scarlet fever had never been reported on the island. Swabs were obtained from the throats of 168 persons, and from the noses of 93. No diphtheria bacilli were isolated, and hæmolytic streptococci were found in only four swabs.

There is great need of further studies in which full bacteriological and immunological tests are carried out on isolated or semi-isolated populations.

Although they are not complete, in this particular sense, the observations of Dungal (1932) on diphtheria in Reykjavik are particularly instructive. From 1926 onwards diphtheria has been very rare, not only in Reykjavik but in Iceland as a whole; but in the preceding years there was a moderate prevalence, reaching its peak in Reykjavik in 1921 with an attack

ABRIDGED CLASSIFICATION OF THE SCHIZOMYCETES

Order

Family

Tribe

Eubacteriales (suborder Eubacterineae)	Nitrobacteraceae	..	Nitrobacteriaceae....
			Hydrogenomonadaceae..
			Thiobacillaceae . . .
	Pseudomonadaceae		Pseudomonadaceae
			Spirillaceae
	Azotobacteraceae	
	Rhizobaceae	
	Micrococcaceae.		
	Neisseraceae		
	Lactobacillaceae		Streptococceae
			Lactobacillaceae
	Corynebacteraceae	..	
	Achromobacteraceae	.	
	Enterobacteraceae	.	Escherichaceae. .
			Erwineae. . .
			Serrataceae .
			Proteace .
			Salmonellaceae
	Parvobacteraceae		Pasteurellaceae
			Brucellaceae
			Bacteroidaceae
	Bacteriaceae		
	Bacillaceae	.	

Actinomycetales

Actinomycetaceae

Chlamydobacteriales
Myxobacterales

Spirochaetaceae

Spirochaetales.

Treponemataceae

experimental evidence is, of necessity, scanty ; but so far as it goes it is demonstrative.

Guthrie, Marshall and Moss (1921) collected 8 volunteers and determined their reaction to the Schick test ; 4 reacted positively and 4 negatively (including 2 pseudo-reactions). The throat of each of these 8 volunteers was swabbed with a virulent culture of the diphtheria bacillus. The four positive reactors developed clinical diphtheria ; the 4 negative reactors, possessing natural antitoxin at or above the Schick-immune level, developed no illness, though 3 of them became carriers.

The main body of evidence on which we rely in concluding that natural antitoxin confers natural immunity has, however, been collected during the course of extensive clinical and epidemiological studies on diphtheria and scarlet fever (see Chapters 61 and 66). There is no doubt that a person whose blood contains antitoxin at or above the Schick-immunity level is far less likely to contract diphtheria than a Schick-positive person. If he does so, the attack will often be mild and will seldom prove fatal. The nature of the evidence that associates natural antitoxic immunity with effective resistance to scarlatinal infection is of the same general kind as in the case of diphtheria.

The Natural Antibacterial Antibodies

It has long been known that there are naturally occurring agglutinins, bactericidins and other sensitizing antibodies, just as there are naturally occurring antitoxins ; but the ground that has been surveyed in the two cases is rather different. We have seen that our knowledge of the distribution and mode of origin of the natural antitoxins is, in the main, derived from the study of diphtheria and scarlet fever in man. Our knowledge of the distribution and mode of origin of the antibacterial sensitizers is, in the main, derived from studies on their occurrence in different species of animals.

One of the earliest studies made was by Burgi (1907), who tested sera from the dog, guinea-pig, rabbit, goose, hen, sheep, goat, horse, ox and man against suspensions of various bacteria, including *V. cholerae*, *Salm. typhi*, *Bact. coli*, *Past. arisepitica*, *Staph. aureus* and *Proteus vulgaris*, and noted well-marked agglutination in many instances, often to a relatively high titre. However, the various sera tended to have much the same order of activity, against whichever species of organism they were tested ; and this order was in general maintained when the sera were tested for their ability to flocculate suspensions of mastic.

Gibson (1930) carried out similar experiments with sera from the ox, rabbit, guinea-pig, horse, sheep, pig, rat, cat and man, tested against *Proteus* X19, *Ps. pyocyanea*, *Salm. typhi*, *Salm. paratyphi A*, *Salm. paratyphi B*, *Salm. enteritidis*, *Sh. flexneri*, *Sh. dysenteriae*, *Proteus morgani*, *Bact. friedlander*, several strains of *Bact. coli* and *V. cholerae*. He found, like Burgi, that the sera tested tended to fall in a definite order with regard to their power of agglutinating a wide variety of bacteria. Ox sera were the most active ; pig and horse sera were somewhat less active ; sheep serum was next in order ; while human, cat, rabbit and guinea-pig sera, in that succession, showed progressively weaker effects. Rat serum was the weakest of all. Similarly the different bacterial species showed a definite order of sensitivity to agglutination. *Sh. flexneri*, *Bact. friedlander* and *Ps. pyocyanea* were the most sensitive, and certain strains of *Bact. coli* the least sensitive. By absorption experiments, this agglutination proved to be dependent on specific antibodies ; but since any one organism, in addition to removing completely the specific agglutinin acting on itself, might reduce the titre of the serum for other organisms, and a similar general reduction in titre might be produced by adsorption with some non-specific agent such as charcoal, he concluded that another non-specific serum constituent was concerned in the

SLIGHTLY MODIFIED FROM BERGEY (1948)

Genus	
Nitrosomonas .	} the nitrifying bacteria (p. 78)
Nitrosococcus . .	
Nitrobacter .	
Hydrogenomonas	the hydrogen bacteria (p. 83)
Thiobacillus .	certain of the sulfur bacteria (p. 80)
Pseudomonas	Ps. pyocyaneus et al (p. 529)
Methanomonas	methane bacteria
Acetobacter .	acetic acid bacteria
Vibrio	cholera, paracholera and non-cholera vibrios (p. 478)
Cellvibrio .	cellulose oxidizing vibrios (p. 86)
Thiospira	sulfur vibrios
Spirillum .	Sp. minus (rat-bite fever) and saprophytic species (p. 752)
Avotobacter	non-symbiotic nitrogen-fixing bacteria (p. 111)
Rhizobium	symbiotic nitrogen-fixing bacteria (p. 113)
Agrobacterium	plant pathogens and saprophytes
Chromobacterium	certain pigmented saprophytic bacteria
Staphylococcus	the staphylococci (p. 346)
Gallicya	M. tetragenus and related bacteria (p. 354)
Sarcina	S. lutea and other cocci
Neisseria	gonococcus, meningococcus, etc. (p. 395)
Veillonella	certain anaerobic cocci (p. 413)
Diplococcus .	pneumococcus and related forms (p. 381)
Streptococcus	the streptococci (p. 355)
Leuconostoc	saprophytic cocci
Lactobacillus	} lactic acid bacteria (p. 531)
Microbacterium	
Propionibacterium	
Corynebacterium	diphtheria and diphtheroid bacilli (p. 606)
Listeria	L. monocytogenes (p. 538)
Erysipelothrix . . .	bacterium of erysipeloid and swine erysipelas (p. 673)
Alcaligenes	Alc. fecalis and related forms (p. 463)
Achromobacter	non pigmented soil and water bacteria
Flavobacterium	pigmented soil and water bacteria
Escherichia	} coliform bacteria (p. 418)
Aerobacter	
Klebsiella . . .	
Erwinia	plant pathogens
Serratia	Bact. prodigiosum and related forms
Proteus	Pr. vulgaris and related forms (p. 428)
Salmonella	typhoid and paratyphoid bacilli (pp. 432, 448)
Shigella	dysentery bacilli (p. 464)
Pasteurella	plague and hemorrhagic septicemia bacilli (p. 502)
Malleomyces	glanders bacillus (p. 599)
Actinobacillus	actinobacillous (p. 672)
Brucella	bacilli of undulant fever and contagious abortion (p. 492)
Bacteroides	non sporulating obligate anaerobes (p. 540)
Hemophilus	influenza bacillus (p. 515), pertussis bacillus (p. 520), chancreoid bacilli (p. 526)
Bacterium	miscellaneous non spore forming bacilli of uncertain status
Bacillus	aerobic, spore forming bacilli such as B. anthracis, B. subtilis, etc. (p. 556)
Clostridium	obligate anaerobic, spore forming bacilli, including bacilli of tetanus (p. 568), gaseous gangrene (p. 575) and botulinus bacillus (p. 591)
Nocardia	aerobic, sometimes acid fast actinomycetes (p. 591)
Actinomycetes	anaerobic actinomycetes of actinomycosis (p. 659)
	filamentous saprophytic bacteria, including sheathed iron bacteria (p. 82)
Spirillum	spore forming saprophytic bacteria having a pseudoplasmodial stage
Spirillum	saprophytic water forms
Spirillum	parasite of molluscs
Torrefila	relapsing fever spirochetes (p. 727)
Treponema	spirochetes of syphilis (p. 733) and yaws (p. 741)
Leptospira	spirochetes of infectious jaundice and Weil fever (p. 746)

only to a few. The specificity of the flagellar agglutinins in normal sera seems to have been clearly established, and the evidence is definitely in favour of the specificity of some, at least, of the agglutinins acting on the antigens at the surface of the bacterial cells. These agglutinins, flagellar and somatic, are, it should be noted, usually present in low titre only ($1/5$ – $1/40$ or thereabouts). The evidence with regard to the specificity of the bactericidal antibodies is more conflicting; but results indicating specificity have been recorded, and if somatic agglutinins are present we should expect them to exert a bactericidal action in the presence of complement. (For examples of natural antiviral antibodies, see Chapter 87.)

The conclusion that the natural antibacterial sensitizers are specific does not, of course, imply any particular hypothesis as to their mode of origin; it assumes only that they unite with certain bacteria because of a physico-chemical correspondence of certain specific combining groups. Moreover, even though an antibody results from an external antigenic stimulus, it may have been formed in response to antigens of an entirely different origin from those in the bacterial suspension used to detect it.

We need not, however, assume that all specific natural antibodies are induced by active immunization. A "natural" antibody and an "immune" antibody, both acting on the same bacterium, must each possess chemical groupings with a specific affinity for groupings borne by the bacterium; but, if the natural antibody has arisen in some way other than in response to infection with the actual bacterium concerned, it is quite possible that the chemical correspondence between its active groupings and those on the bacterial surface will be less close than in the case of an antibody produced in response to infection or artificial immunization. Certain experimental findings are consistent with such a possibility. It is, for instance, a very general experience that some, at least, of the "natural" antibodies require, for their removal from a serum, an absorbing dose of bacteria very much larger than that required for the removal of the same amount of an "immune" antibody.

If we adopted this view we should expect that the antibacterial antibodies demonstrable in any random sample of a human or animal population would be of two kinds, those arising as the result (*a*) of contact, whether infective or not, of the tissues with the bacterium on which they act, or with other, though related, antigenic material, and (*b*) those arising in some other way. This brings us directly to the question of their origin under natural conditions.

Some Examples of Natural Agglutinins.

It is, of course, well established that certain kinds of "natural antibodies" are produced in the absence of any environmental stimulus, and in accordance with definite genetic laws.

The best example is provided by the isohæmagglutinins that divide mankind into different blood-groups.

Landsteiner (1901) distinguished the principal blood-groups into which the human race may be divided, and showed that this grouping depends, in the main, on the distribution of two different antigenic constituents, which may be present together, or separately, or may both be absent. There are two corresponding antibodies, and these are so distributed that neither antibody is present in the blood of an individual whose red cells contain the corresponding antigen. By determining the antigens present in any sample of blood it is therefore possible to assign it to its correct group. The antigens are referred to by a capital letter, the antibodies by Greek letters, and the blood-group

down into genera other than *Hemophilus*. These and minor variations which appear later have been retained to keep the nomenclature from differing too widely from practice in other than current American literature. For example, the name *Vibrio comma* for the cholera vibrio is not used outside the Bergey classification; over 98 per cent of the papers published on this bacterium in the past 20 years are not American, and consistently use the name *Vibrio cholerae*. Abridged keys for the differentiation of the better known species of bacteria are included in subsequent chapters.

Nomenclature.⁵ Throughout the development of bacteriology names have been given these organisms in a haphazard fashion and often with singular disregard for the conventions of botanical nomenclature. In general, however, a bacterium has a generic name, always written with a capital letter, which may or may not be descriptive (for example, *Bacillus*—a small rod; or *Pasteurella*—in honor of Pasteur) and a specific name which may be an adjective (*albus*—white) or a noun indicating possession (*Clostridium welchii*—Welch's clostridium) or a noun in apposition (*Bacillus radicolola*—the root-dweller bacillus). The practice of indicating the author of the name, as in *Bacillus subtilis* Cohn, is not as common in bacteriology as in zoology or botany. The use of trinomial and quadrinomial names such as *Granulobacillus saccharobutyricus mobilis nonliquefaciens* is obviously highly undesirable. Trinomials are occasionally useful in the designation of varieties of subspecies. Common names are, of course, used; terms such as Friedländer's bacillus, the typhoid bacillus, etc., are frequently encountered.

Genera. Perhaps one of the greatest difficulties the bacterial taxonomist labors under is the paucity of genera. The question of what degree of difference shall be judged sufficient to establish new genera is one to which there is as yet no satisfactory answer. At the present time considerable confusion and lack of uniformity still exist. In a number of instances new generic names have obtained wide currency among bacteriologists in many parts of the world, partly, doubtless, because they are applied to fairly distinct groups of microorganisms and have genuine classificatory value. Such are *Brucella* (for the bacilli of undulant fever of man and contagious abortion of cattle), *Salmonella* (for the paratyphoid bacilli) and *Pasteurella* (the bacilli of hemorrhagic septicemia in domestic animals and the bacilli of plague and tularemia); the name *Shigella* for the dysentery bacilli has gained a considerable degree of recognition. Without any formal international agreement, these names can be said to have won international standing.

In a number of instances it seems necessary to mention both old and new names whenever a microorganism is referred to. Few bacteriologists venture to use the name *Serratia marcescens* without explaining that they mean *Bacterium prodigiosum* or *Bacillus prodigiosus*, or *Gaffkya tetragenus* without the equivalent *Micrococcus tetragenus*. Similarly the adherence of bacteriologists to various taxonomic schemes gives rise to a series of names for a microorganism, the familiar typhoid bacillus may be *Bacterium typhosum*, *Bacillus typhosus*, *Salmonella typhi*, *Eberthella typhosa* or *Salmonella typhosa*.

For the present the bacteriologist seems to have no escape from using a

⁵ Rules of nomenclature, proposed but not officially adopted, are discussed by Buchanan, St. John-Brooks and Breed Jour. Bact., 1948, 55:287.

British and white American people, about 85 per cent. of the population are Rh-positive, the remainder Rh-negative. The Rh-positive persons, are in fact those who, among the 8 sub-types of the Rh system, possess the D antigen. When an Rh-negative woman mates with an Rh-positive man, the foetal erythrocytes may be Rh-positive, and in a certain number of cases, depending perhaps on the condition of the placenta, the Rh antigens of the foetus are transferred to the mother, and induce the formation of Rh-agglutinins. Towards the end of pregnancy, these agglutinins appear in the circulation of the foetus, and, uniting with the foetal erythrocytes, sensitize them to lysis, in a manner analogous to the sensitization, let us say, of Group A cells transfused into a Group B person with natural α agglutinins in his serum. The resulting destruction of the red cells may lead to the death of the foetus and abortion, or the birth of an infant with the syndrome known as erythroblastosis foetalis (Levine, Burnham, Katzin and Vogel 1941). Certain other of the Rh antigens, but not all of them, are capable of inducing hæmolytic disease. We have in this disease a clear-cut example of uterine transference of foetal antigen to the mother, and then of maternal antibody to the foetus. The Rh antibody may also be transmitted by the colostrum. There is clearly a difference between the action of maternal Rh-agglutinins and the maternal α and β agglutinins, for it is only rarely that serious blood destruction occurs in infants of the A, B, or AB groups whose mother's blood contains agglutinins for A, B or AB cells. We may postulate a selective transmission of Rh, but not α or β agglutinins; or that the infants are insusceptible to α and β agglutinins (see, e.g., Tovey 1945); or the agglutinins are more quickly eliminated from the infant's circulation (Wiener 1951a). It has been suggested that the cells of the infant are protected from α and β agglutinins of the mother by water-soluble Group A or B substances which are present in the tissues and body fluids of the infant, and which unite with any maternal agglutinins that reach the foetus. Group A and B cells are characterized by viscous mucopolysaccharides (see the next section), which occur in a water-soluble form in the tissues and body fluids of most persons of the corresponding blood-group—e.g., in the saliva, gastric juice and in the secretion of pseudomucinous ovarian cysts. Their presence in the body is determined genetically by an independent "secretor" gene, S. The Rh substance, on the other hand, is confined largely to the erythrocytes (Levine and Katzin 1941, Wiener and Forer 1941) and, being absent from the tissue fluids, is not available for the neutralization of maternal Rh-agglutinins. (For a full discussion, see Levine 1943.) The validity of this explanation is not yet established.

Apart from the direct hazard of transfusion with cells for which the recipient's serum may contain natural isohæmagglutinin, there is the less direct but equally real hazard of transfusion reactions in persons whose serum, as a result of previous transfusion, or, in women, of pregnancy with a foetus of a different blood-group, contains hæmolysins for the donor's cells. As with hæmolytic disease, these reactions are commonly due to Rh agglutinins, and to antibodies for antigens of the systems known as Kell, Duffy and Kidd; though on rare occasions other antigens are responsible.

The hæmagglutinins so induced usually differ from iso-agglutinins in reaching higher titres, which tend to decline when the stimulus ceases; in reacting with R.B.C. better at 37° C. than at room temperature; and in a greater heat resistance. Moreover, though they may be detectable by the orthodox agglutination technique in 0.85 per cent. saline, they are commonly present as inapparent agglutinins, requiring special techniques for their demonstration (see Chapter 7). They also appear to pass readily from mother to foetus, and are important in hæmolytic disease. The titre of α - and β -iso-agglutinins, on the other hand, is low and tends to be constant. The antibodies are most active at room temperature, and are relatively heat-labile; they are not important in hæmolytic disease. The distinction is not always clear cut, but it is sufficiently striking to lend colour to the view that the "natural" hæmagglutinins are produced differently from "immune" hæmagglutinins; and are the result of "serogenesis". On the other hand, their appearance after birth, and the widespread occurrence in nature—in the saliva of secretors, in other mammals and mammalian foodstuffs, in plants and microbes (see next section)—of antigens

double set of generic names for certain organisms, or from using the older, albeit nomenclatorially unjustifiable, names for others, while welcoming certain new and useful generic designations such as *Brucella* and *Salmonella*. Uniformity, consistency and strict adherence to the rules of biological nomenclatorial practice are perhaps in the future.

Species. The differentiation of species is made for the most part on a physiological basis. A single character is hardly sufficient, particularly in view of the facility with which variation occurs. The use of a number of such characters is the rule, but difficulties are frequently encountered when intermediate forms occur. For example, *Bact. coli* and *Bact. aerogenes* are almost universally regarded as different species and in the Bergey classification are put into different genera, *Escherichia* and *Aerobacter* respectively. Yet forms intermediate between the two are so commonly encountered that it sometimes seems a doubtful procedure to attempt to separate these so-called coliform bacteria.

Types. Immunological differentiation is generally regarded as not of species status though it may sometimes coincide with species differentiation on a physiological basis. Conversely, minor cross reactions between species are not indicative of species identity. There are some exceptions to the former, however, and many workers divide the genus *Salmonella* into species on the basis of antigenic structure. Immunological differences are of very considerable value, particularly for epidemiological purposes, and are usually the basis of distinction of types within a species. Thus the streptococci are divided into groups, designated A, B, C, etc., on the basis of one kind of antigen, and into arabic numbered types within and across species by another. The pneumococci are similarly divided into numbered types on the basis of capsular antigen. *Clostridium botulinum* is separated into types, A, B, C, etc., on the basis of the immunological specificity of the toxin, and these have no relation to the immunological character of the cell substance. There is, then, no general practice with respect to type differentiation. Physiological differences within species are usually not used to differentiate types, and the biochemical reaction is simply given as variable.

stimulate the production of heterophile antibody; and in general it appears that those species that contain heterophile antigen in their red corpuscles do not contain it in their tissues, at least in the full and effective antigenic form.

It soon became clear, from absorption experiments of the usual type, that the hæmolysin produced by the rabbit in response to guinea-pig's kidney was not identical with that produced in response to sheep's corpuscles, which is a mixture of hæmolysins, only one of which corresponds to the heterophile antigen. (See, e.g., Tomcsik and Fisher 1946, Tomcsik and Schwarzweiss 1948.)

Only animals of the rabbit type—those not containing heterophile antigen in their tissues—are capable of producing heterophile antibody in response to guinea-pig's kidney or other suitable tissue. It is also of particular interest that certain normal antibodies are of the heterophile type—for instance, the natural hæmolysin for sheep's red cells that is found in the serum of rabbit and man (Friedemann 1917). In human serum, this reaction may depend on the α -iso-agglutinin, for, as Schiff and Adelsberger (1924a b) showed, there is a close antigenic relation between the heterophile antigen and the Group A substance.

The constitution of the heterophile antigen appears to vary with the organ in which it is found. According to earlier workers (Iwai 1917, Georgi 1919, Taniguchi 1921) the specific component (hapten) is associated with lipid constituents of the tissues, especially the alcohol-soluble "lecithin" fraction. The heterophile antibody, in addition to acting as a hæmolysin for sheep cells, forms a precipitate and fixes complement when mixed with an emulsion of these lecithin fractions of tissues containing heterophile antigen. The lipid extracts are not antigenic, but can be made so by mixing with a suitable protein, the resulting antisera containing both heterophile antibodies and antibodies specific for the protein used (Landsteiner and Simms 1923). These haptens were apparently lipopolysaccharide in nature (Landsteiner and Levine 1925, 1927; but see also Renaux and Thomas 1942).

The Group A substance, which in pure form is non-antigenic in the rabbit (i.e., a hapten), has proved to be a viscous substance made up of polysaccharide units, partly hexose, partly N-acetylglucosamine, and a number of amino-acids (Goebel 1938, Landsteiner and Harte 1940, 1941, Morgan and King 1943, King and Morgan 1944, Aminoff, *et al.* 1950).

This substance, or the mucopolysaccharides closely resembling it, is found in mammals other than man—for example, in the gastric mucosa of swine, cow and horse. Substances either identical with or very like the B substance, and the H substance found in Group O cells, are also present in the secretions of other mammals; they have been extensively studied by Morgan and by Kabat, whose reviews should be consulted by those desiring detailed information (Kabat 1949, 1952, Morgan 1953). A remoter instance of an antigenic relationship of the A, B and O substances is provided by Type XIV pneumococcus; horse antisera prepared against this organism agglutinate the cells of all four major blood-groups. All three substances contain L-fucose, D-galactose and N-acetyl-D-glucosamine; and the Type XIV pneumococcal polysaccharide contains D-galactose and D-glucosamine. Cross-reactivity of the blood-group substances with this antiserum is greatly increased by a degree of hydrolysis which destroys blood-group specificity—a procedure which removes the L-fucose residues (see Kabat 1952).

✓ As we have indicated in Chapter 8, a number of bacteria contain the Forssman antigen or substances immunologically related to it. In *Sh. dysenteriae*, the Forssman hapten is associated with the lipopolysaccharide moiety of the somatic antigen (Meyer and Morgan 1935, Meyer 1938, Morgan and Partridge 1940, Davies and Morgan 1953, Davies *et al.* 1954). As Morgan showed, the hapten from *Sh. dysenteriae* is not antigenic, but can be made so by reunion with the protein moiety of the antigen (see p. 303). The Group A substance can in the same way be made powerfully antigenic by union with this *Sh. dysenteriae* protein; and the resulting antigen, like the complete antigen of *Sh. dysenteriae*, stimulates the production of heterophile antibodies. This interesting demonstration, together with that of Landsteiner and Simms (1923), suggests a means whereby heterophile

THE RELATION OF BACTERIA TO DISEASE

Prior to the discovery of the causal relation of bacteria to the infectious diseases, these afflictions were regarded as an outward manifestation of the activity of some metaphysical or supernatural agency. A common belief, and one that still persists among some primitive peoples, was that of demoniacal possession, the invasion of the human body by the demon, taking the form of punishment for misdeeds or a consequence of individual failure to take adequate precautions against malignant spirits. When the advance of knowledge brought a larger measure of understanding of the structure and functions of the human body, a new and semiscientific theory of disease sprang into being and, although not entirely displacing the concept of demoniacal invasion, attained world-wide influence. The Hippocratic theory of disease, as it was called after its founder, the Greek physician Hippocrates, postulated four bodily humors, blood, phlegm, yellow bile and black bile. Health consisted of a proper mixture of these humors, disease an improper mixture. Although supplanted to a mild degree in the seventeenth century by more complex and mystical theories such as the homeopathy of Hahnemann, the Hippocratic doctrine of humors was dominant throughout the middle ages and even yet colors much medical thought and practice.

Amid the vagueness and confusion of these half mystical hypotheses emerged the tangible and definite germ theory of disease. As already pointed out, the germ theory of disease is the legitimate offspring of the germ theory of fermentation, and owes its origin to the memorable investigations of Louis Pasteur. The belief that the infectious diseases are caused not by demons or improper mixtures of humors or by any spiritual dynamic derangement but by microscopic plants and animals is now securely established on a firm experimental foundation.

✓**Koch's Postulates.** The unequivocal proof of a suspected causal relation between a given bacterium and a particular disease is dependent upon the development of a logical chain of experimental evidence which is often formalized as a series of postulates. These are commonly known as Koch's postulates although there is no evidence that Koch himself expressed these experimental steps in terms of formal "postulates." There are four of these:

- (1) The bacterium must be observed in every case of the disease.
- (2) The bacterium must be isolated and grown in pure culture.
- (3) The bacterium, in pure culture, must, when inoculated into a susceptible animal, give rise to the disease.
- (4) The bacterium must be observed in and recovered from the experimentally diseased animal.

specificity that proves to be based on an accidental correspondence of the surface groups of a mammalian serum protein with those of a substance characterizing a bacterial species—a correspondence apparently not induced by the stimulus of a related antigen. It illuminates the problem of the origin of natural antibody inasmuch as it indicates the possibility of an "antibody" formation that is independent of stimulation by the kind of antigens we discussed in Chapter 7. It may also be independent of the mechanism of antibody formation discussed in Chapter 50. However, in the rabbit immunized with human serum proteins Wood (1953) demonstrated the early production of Cx-reactive protein, and an association between its titre and that of the specific antibodies subsequently produced. Certain insoluble adjuvants to antibody formation (see Chapter 50) also induced its appearance. The Cx-reactive protein presumably comes from tissues newly formed or newly responding to an external stimulus; whether it comes from cells that later will produce specific antibody remains to be determined.

Heterophile antigens and antibodies other than those of the Forssman type occur in animal tissues. Thus Deicher (1926) records that the injection of horse serum into man induces the formation of agglutinins not only for sheep erythrocytes, but also for those of the rabbit, which contain no Forssman antigen. The distinction of these agglutinins from Forssman antibody was confirmed by Schiff (1937) who demonstrated them in man following injection of rabbit serum, which also contains no Forssman antigen. There appeared to be two antigens concerned, one specific for rabbit erythrocytes, the other shared with those of the sheep, horse and ox. Graña (1944) records similar antibodies in patients with hydatid cysts who have been injected with hydatid fluid. Brown (1943) found an additional thermolabile antigen in mouse tissues distinct from, but related to, the Forssman antigen. (For further information about heterophile antigens, particularly in relation to polysaccharide substances, see the review by Tomcsik 1945.)

Age and Natural Antibody Production.

Excepting those passively derived from the mother, "normal" hæmolysins and hæmagglutinins are absent from the blood of new-born infants and of other very young animals, and are developed during the process of normal growth (Hirszfeld 1926, Friedberger, Bock and Furstenheim 1929). The same is apparently true of the natural antibacterial agglutinins (Kraus and Low 1899, Ludke 1905, Braun 1909, Gibson 1930; see also Mackie and Finkelstein 1928, 1930). Both Rywosch (1907) and Sherman (1919) noted that the normal hæmagglutinins of the fowl did not appear in the chick embryo until the twenty-first day, though Rywosch observed lysins of *Bact. coli* as early as the fourteenth day. In this connection it may be noted that Polk, Buddingh and Goodpasture (1938) could find no complement in the chick embryo. It appeared suddenly in the newly hatched chicken, and gradually increased with age. Observations made by Bailey (1923) add a point of considerable significance. He also noted the absence of hæmagglutinins in the young chick, and their appearance during the normal process of growth, but he also found that chickens failed to respond to immunization with foreign red cells until they had spontaneously developed some agglutinating capacity (see also Wolfe and Dilks 1948). The inability of the antibody-forming apparatus to respond effectively to an antigenic stimulus during the earliest period of life would seem to be a general rule. Thus, Blum (1932) and others have noted the failure of young infants to produce diphtheria antitoxin in response to the injection of toxoid. The antibody response of young rabbits to the injection of bacteria, erythrocytes, or of soluble protein antigens is poor (Freund 1930). Baumgartner (1934) found that production of agglutinins to *Salmonella enteritidis* given intravenously to rabbits was not maximal until maturity was reached. The response in 50-day-old rabbits was poor, and moderate at 70 days. It is also noteworthy that antibody from the younger animals had a lesser "avidity" than that from the mature rabbit. Baumgartner (1937) later observed a similar age difference in the response to ovalbumin, and showed that the combining power of the precipitins produced increased with the age of the immunized animal. Although it may be generally true that the capacity of very young animals to produce

Clearly, if each of these steps can be carried out, the evidence implicating the bacterium as the causative agent of the disease is very strong indeed.

Although in a great many cases this chain of experimental evidence can be developed, or, as sometimes said, Koch's postulates can be fulfilled, and the story is in its essentials complete, in a considerable number of diseases one or more of these steps cannot be carried out. In this connection, these experimental steps are best considered one by one.

The *first postulate* may be fulfilled in practically all cases if the term "observe" may be taken in its broad sense. Bacteria, of course, can be observed in the literal sense, but the submicroscopic filterable viruses cannot be seen with the aid of ordinary equipment. Their presence may be demonstrated, however, by animal inoculation, and it is not unreasonable to regard such a procedure as observation in a very real sense. It should be pointed out here that in the early days of bacteriology it was assumed that the bacterium under suspicion should be present only in cases of disease and not in healthy individuals. It is now well known that the corollary does not necessarily follow. A number of pathogenic bacteria, such as those causing diphtheria, typhoid fever and the like, may be present in a virulent form in healthy persons who show no clinical symptoms.

The *second postulate* is, in general, somewhat more difficult to satisfy, although in most cases a causative bacterium may be isolated and grown in pure culture. Perhaps the best known example of an organism that has not been cultivated outside the body of the host is the leprosy bacillus (see, however, p. 647), and as a consequence it cannot be said with certainty that this bacterium is responsible for the disease. The ultramicroscopic viruses have not been cultivated on lifeless media and, although these agents may be shown to proliferate in tissue culture or in the developing chick embryo (p. 850), there is no positive assurance that such cultures are "pure," i.e., that a virus is but a single entity and not a mixture of two or more viruses.

The *third postulate* is, in practice, probably the most difficult one to fulfill. The disease must be reproduced in a clinically recognizable form, a localized infection or a general septicemia is not sufficient although often suggestive. Since the most important diseases, from the anthropocentric point of view, are the diseases of human beings, it is necessary to find an experimental animal that will respond to the infection in the same or nearly the same manner as the human subject. A satisfactory experimental animal which complies with this requirement may be difficult to find, for phylogenetic relations are not necessarily correlated with susceptibility; monkeys, chimpanzees and the like are not, in general, better experimental animals than rabbits, guinea pigs, etc. The importance of the experimental animals to the study of an infectious disease is obvious, epidemic influenza, for example, in spite of the tremendous amount of concerted study which followed the 1918 epidemic, was but poorly understood until it was discovered (1933) that the disease could be reproduced in the ferret. The difficulties sometimes encountered in the experimental reproduction of a disease very likely stem in part from the adaptation of the bacterium to a parasitic mode of existence in conjunction with a particular host species.

The *fourth postulate* generally offers no difficulties if the preceding three

This conclusion does not, however, apply to all kinds of natural antibodies. Though the greater part of the evidence bearing on this problem has been obtained by studies of the kind outlined above, some of it has emerged from surveys of the normal antibodies present in the sera of human groups living under different environmental conditions. Thus Silverthorne (1936) determined the nasopharyngeal carriage of meningococci in a group of human subjects, and found a high degree of correlation between the bactericidal capacity of the blood for meningococci and their presence in the nasopharynx. It has sometimes been possible to correlate the results of such surveys with the associated epidemiological records, as in the studies on the distribution of diphtheria, or scarlatinal antitoxin referred to above (see, however, Peters 1940); and it has been shown quite clearly that the frequency of natural agglutinins for a particular bacterium, among a random sample of the human population living in a particular locality, is closely associated with the frequency of infections due to that bacterium in that locality. Examples are provided in the epidemiology of enteric infection (Chapter 69), of undulant fever (Chapter 75), and of certain virus diseases (Chapter 87 and 88).

A few surveys of a similar kind have been made in laboratory animals. Thus Bailey (1927) examined 81 rabbits taken from a stock in which cases of snuffles were occurring due to *Pasteurella leipsetica*. This organism was isolated from the nose, or from abscesses in three rabbits with acute snuffles, nine with chronic snuffles, three with suppurative lesions, and six with no symptoms of infection; all had agglutinins and complement-fixing antibodies in the serum. On the other hand, ten rabbits with no symptoms of infection and no detectable *Past. leipsetica* in the nares had no serum antibody. *Past. leipsetica* was instilled into the nose of five of these animals. All became symptomless carriers of the organism, and antibody appeared in the serum during the succeeding three months.

There is then no doubt that the members of a herd, or population, submitted to a constant or recurrent risk of infection with a particular bacterium will tend to form antibodies acting on that particular organism in response to the stimuli provided by infection, whether manifest or latent; and the natural working of this mechanism will certainly play a large part in determining the frequency distribution of the antibodies concerned.

We must then accept the view that the naturally occurring antibacterial sensitizers, and perhaps also the natural antitoxins, may owe their origin to any one of four mechanisms: actual infection with the corresponding bacterium; infection with some other organism that shares a common antigenic component; the entrance to the tissues *via* the intestinal tract, or possibly by other routes, of antigenic material capable of stimulating the production of an antibody with the active group in question, or the formation of such antibodies as a by-product in the normal functioning of the antibody-forming apparatus altogether apart from any specific external stimulus.

The Immunological Significance of the Natural Antibacterial Antibodies.

Do naturally occurring antibodies of the type considered above confer a relative immunity to the risk of natural infection, analogous to that conferred by the natural antitoxins? The evidence available does not allow of any but the most tentative answer. So far as it goes it suggests that there is sometimes a relation between the presence in the blood of a particular antibacterial sensitizer and a relatively high resistance to the corresponding bacterium; but that this relation

can be satisfied. Its importance should, however, not be minimized, for the presence of the microorganism in the experimentally infected animal is indicative of its proliferation and invasion of the host tissues.

A fifth postulate is sometimes added to the original four, namely: the injection of the products of a bacterium should give rise to the clinical symptoms of the disease. This postulate is applicable to those organisms which form soluble or exotoxins, such as the diphtheria and tetanus bacilli, but it is not of general significance in the incrimination of a bacterium as a cause of disease.

As indicated above, not infrequently one or more of these postulates cannot, or has not, been satisfied with respect to a given disease. The question then arises as to whether the suspected microorganism may be held responsible for the disease on the basis of only partial compliance with Koch's postulates. There is, unfortunately, no general answer to this; each case must be considered on its own merits. In some diseases, such as leprosy, the actual evidence consists in the observation of an association of a particular bacterium with a certain clinical syndrome and pathology. Whether the association may be interpreted in terms of causality is, of course, quite uncertain and consequently the etiology of leprosy is, as noted above, not definitely known. The absence of other possible etiologic agents may be regarded as favorable, though weak, ancillary evidence, and *Mycobacterium leprae* is generally, though tentatively, considered to be the cause of this disease.

In other cases indirect evidence assumes a more important position; in that of the virus diseases, for example, while there is no absolute assurance that a tissue culture represents a pure culture of virus, the homogeneity of the infective agent is rendered highly probable by facts such as the immunological relation existing among strains of a virus (e.g., influenza or poliomyelitis) and the relative constancy of the epidemiology and pathology of the disease. The inability to isolate a filterable virus in "pure culture," then, is not a serious deterrent to the implication of these agents in the causation of disease.

The case of typhoid fever is somewhat different, for here, although the organism may be grown in pure culture, the clinical picture of the disease cannot be reproduced in the laboratory animal. Indirect evidence such as the epidemiology of the disease, the undoubted efficacy of immunization with killed suspensions of the typhoid bacillus in pure culture, etc., is so strong that the disease may be regarded as almost certainly caused by this microorganism, even in the absence of the inadvertent laboratory infections of human beings which have supplied the missing link in the chain of evidence.

It is clear, then, that while the fulfillment of Koch's postulates is, in general, essential to the proof of the bacterial etiology of a disease, in some instances in which a part of the necessary direct evidence is lacking the weight of ancillary evidence may be sufficient to make the postulated causal relation highly probable or, occasionally, practically certain. It may be noted at this point that the logical processes contained in Koch's postulates are by no means confined to the study of infectious disease, the responsibility for a given fermentation, for nitrogen fixation, etc., is fixed upon a particular microorganism by essentially the same logical development of experimental evidence.

Even though a bacterium has been shown to be pathogenic, i.e., capable of producing disease, the outcome of a chance contact between such a micro-

species are relatively susceptible to infection with some of these *Salmonella* spp (Lovell 1934). A particularly striking example of this kind of discrepancy is afforded by the observation that the great majority of normal horse sera agglutinate the glanders bacillus to high titre, though glanders is bacteriologically a disease of equine animals (see Wilson 1934).

SUMMARY

(1) Antibodies of various kinds—antitoxins, agglutinins, bactericidal antibodies and so on—are frequently found in the serum of normal men and animals. The origin of these naturally occurring antibodies is still a matter in dispute; but it seems possible that they may arise in several different ways.

(2) The new-born animal, when born of a naturally immune mother, is endowed with a congenital passive immunity, due to the uterine transference of specific antibodies from the maternal blood to the foetus, or transference to the new-born animal by way of the colostrum during the early days of suckling. This, like other types of passive immunity, is of relatively short duration.

(3) Young animals from whose blood these congenitally acquired antibodies have disappeared and young animals in whom they are not present at birth often develop antibodies with advancing years.

(4) This production of antibodies is dependent on the activity of an antibody-forming apparatus that is imperfectly developed at birth, but reaches functional maturity at a relatively early period of life.

(5) There is insufficient evidence to decide whether certain natural antibodies—like the normal hæmagglutinins and hæmolysins which distinguish one animal species from another, and different varieties within a species—are produced without any external antigenic stimulus. The genetic evidence for an exclusively intrinsic determination of these antibodies is suggestive, but it is not conclusive and our biochemical insight into the processes of such determination is negligible. On the other hand, our ignorance of all but a few of the undoubtedly numerous antigenic hazards to which a mammal is subject makes it difficult to deny the existence of an external antigenic stimulus in cases where no such stimulus has been discovered.

It remains possible that certain of the antibodies acting on bacteria or their products may be formed by a process of normal serogenesis, determined ultimately by genetic factors.

(6) The more commonly accepted view is that these antibodies arise as the result of the response of the antibody-forming apparatus to external environmental stimuli, and that such stimuli usually consist of overt or latent infections with the bacterium in question. This view is particularly well established in relation to natural antitoxic immunity against diseases like diphtheria and scarlet fever.

(7) In the case of specific antibacterial immunity there are greater difficulties in accepting this hypothesis in its entirety. Samples of sera collected from a given animal species may contain antibodies active against a large number of different bacteria, and it is difficult to believe that a single animal can have been infected with so many different organisms, some of which are not known to be natural parasites of the species to which it belongs. There are, however, good reasons for believing that some at least of the agglutinins or other antibodies found

organism and a prospective host is variable. The result of this contact may be negative with no clinically apparent departure from the normal physiological state regarded as health, or obvious disease of varying degree of severity may ensue. The nature of the outcome is dependent upon the interaction of two factors, the virulence of the organism and the resistance of the host. Both virulence and resistance are vague terms which express a combined effect of a variety of component factors. Some of these are known in each case, although the manner in which they function is generally not well understood, and there are undoubtedly many more which are unknown and possibly unsuspected at the present time.

VIRULENCE

The precise meaning of the term virulence is difficult to define in an entirely satisfactory fashion. Some workers include the ability to form soluble toxins as a part of the virulence of a bacterium, toxigenic diphtheria bacilli, for example, are said to be "virulent" while the atoxigenic diphtheroids are regarded as "avirulent." Others assume virulence to be synonymous with invasiveness, the ability of a microorganism to invade the tissues of the host. Under the circumstances it is perhaps best to disregard these more subtle distinctions and to define virulence as the ability of a bacterium to produce disease, thereby making virulence and pathogenicity synonymous.

The Measurement of Virulence. By definition virulence can be measured only in terms of the production of disease. In man it is usually judged on the basis of case fatality rates, a highly virulent microorganism producing death in a large proportion of the infected persons, and one of low virulence only a small number of deaths. Similarly, the titration of virulence (or any lethal agent such as a toxin) in the experimental animal is based on deaths, but dosage, which is inversely related to virulence, can be varied. Thus virulence (or toxicity) may be defined as the dose which will produce the specified deaths in a specified animal.

In the earlier studies virulence was measured as the minimum lethal dose, defined as that amount of the substance to be tested which would just kill the experimental animal, and definitions of toxin units, etc., are given in terms of these limits (p. 288). In recent years it has become generally apparent, however, that an accurate measure is essentially statistical in nature. It is assumed, and not unreasonably, that the resistance of the normal animal to the lethal agent rests on a probability basis and is normally distributed, i.e., is described by the Gaussian frequency distribution. If this function is integrated, an S shaped curve is given by the plot of the integral which describes accumulated deaths plotted against dosage. The point of inflection is, of course, that dose with which 50 per cent of the animals die. This is called the *median lethal dose* or LD_{50} dose. This dose can be calculated by a relatively simple procedure¹ from the accumulated deaths from graded doses extending through the 50 per cent end point.

For purposes of comparison between, for example, normal and treated animals, determination of the LD_{50} doses allows a relatively precise measure having a standard basis. A somewhat less satisfactory but comparable pro-

¹ Reed and Muench. Amer. Jour. Hyg., 1938, 27: 493.

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cedure is that of relative survival times. A variety of other methods of analysis may be used for purely comparative purposes. For example, control and experimental death rates may be tested for statistical significance by the point binomial or fourfold table. Comparative death ratios, such as 8/10 to indicate that 8 animals died of 10 inoculated, are almost useless from the quantitative point of view.

Toxins. It is difficult if not impossible to account for the derangement of the normal physiological processes and attendant clinical symptoms of an animal suffering from an infectious disease on the basis of a purely mechanical effect of the presence of the invading bacteria in the blood or other tissues. It was early discovered that a number of the pathogenic bacteria produce poisonous substances which, when absorbed by the host, give rise to the symptoms and pathology characteristic of the particular disease. It has been pointed out previously (p. 124) that these poisonous substances or toxins fall into two categories. (a) the *soluble toxins*, *exotoxins* or *true toxins* which apparently diffuse out of the intact bacterial cell into the surrounding culture medium or tissue, and (b) the *endotoxins* which do not diffuse out of the intact bacterial cell but may be separated from it *in vitro* by mechanical disruption of the cell structure. These substances are liberated *in vivo* through destruction of the bacterial cells by the various defensive mechanisms of the host.

The soluble toxins, although not a part of the cell substance, cannot be regarded as secretions in the usual sense of the word. The rate of appearance of toxin does not, except in the case of *Clostridium welchii*, parallel the rate of growth of the culture, toxin is liberated in the greatest amounts after the phase of active growth is over and many of the cells are dead or dying. The endotoxins appear to be a part of the cell substance of bacteria containing them; organisms such as the cholera vibrio, certain of the dysentery bacilli and the like may be regarded as microorganisms whose protoplasm is toxic to the higher animals which they infect. There are a number of differences between these two types of toxins which may be summarized as follows:

Exotoxins

- (1) Occur outside the bacterial cell
- (2) Poisons of extremely high potency.
- (3) Excellent antigens which give antiserum of higher titer.
- (4) Combine with antibody according to the law of multiple proportions.
- (5) Protein in nature.
- (6) Thermolabile.
- (7) Destroyed by proteolytic enzymes.
- (8) Detoxified by formaldehyde.

Endotoxins

- (1) Intracellular in the intact cell.
- (2) Low potency.
- (3) Poor antigens, antiserum of low potency.
- (4) Do not combine in multiple proportions. ✓
- (5) Some are proteins but some are glucolipoids. ✓
- (6) Thermostable.
- (7) Resistant to the action of proteolytic enzymes
- (8) Toxicity not affected by formaldehyde.

These differences are by no means absolute, and the listing of them represents a generalization to which there are a number of exceptions, as will appear. Consequently, the identification of a toxin as an exo- or endotoxin can be made, not on the basis of a single property, but only on the aggregate of the properties it exhibits. Even when all its properties are taken into consideration, a given

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toxin, such as the Shiga dysentery bacillus toxin, for example, may occupy an anomalous position.

The soluble bacterial toxins are, by a comfortable margin, the most potent poisons known, only the vegetable poisons, ricin and abrin, approach them in potency. The most potent of the soluble toxins are those formed by *Clostridium botulinum*, *Clostridium tetani* and the diphtheria bacillus. Crystalline type A botulinum toxin (p. 593) is the most potent of the bacterial toxins, the LD₅₀ for the mouse is only 4.5×10^{-9} mg. N. Type B botulinum toxin is somewhat less potent, 5 to 9×10^{-9} mg. N/LD₅₀ for the mouse, and crystalline tetanus toxin 5 to 7.5×10^{-7} mg. N/LD₅₀. Highly purified but non-crystalline preparations of diphtheria toxin kill guinea pigs in amounts of $0.4 \mu\text{g.}$ per kilo body weight. While these amounts seem fantastically small—for example the LD₅₀ of crystalline type A botulinum toxin is the equivalent of 2.1×10^7 molecules—the effective concentration in the body is much higher in that these toxins have marked affinities for certain tissues, as that of botulinum and tetanus toxins for the nervous tissue. Other toxins, such as that of *Clostridium welchii*, are much less potent, and it may require as much as 0.1 ml. of culture filtrate to kill an experimental animal. Still less potent are the endotoxins whose minimum lethal dose may be as much as one million times greater than that of a potent exotoxin. The MLD of a killed broth culture of the cholera vibrio, for example, is about 0.5 ml. for the guinea pig.

The potency of these toxins is, in general, paralleled by their efficiency as antigens. Antitoxic sera of high titer, 1 ml. of which will neutralize thousands of guinea pig MLD's, may be obtained against diphtheria toxin, but antitoxic sera prepared against endotoxins are generally of very low titer—several milliliters may be required to neutralize 1 to 5 MLD's of endotoxin. The neutralization of exotoxin by antitoxin proceeds according to the law of multiple proportions, i.e., if x units of antitoxin neutralize y units of toxin, nx units of antitoxin will neutralize ny units of toxin (see p. 288). The neutralization of endotoxin does not proceed in this orderly manner; it may be possible to protect an animal against 4 to 5 MLD's by the injection of antiserum, but if much more endotoxin is given, greater amounts of antiserum will not provide protection.

The nature of toxins, the soluble toxins in particular, has been a subject of long-continued interest.² The soluble toxins appear to be proteins; they are denatured by heat, may be salted out of solution, etc. Highly purified diphtheria toxin has been prepared by Eaton³ and by Pappenheimer⁴ and probably represents the pure toxin, the preparations appear to be homogeneous protein. Type A toxin of *Clostridium botulinum* has been prepared in crystalline form by Lamanna, McElroy and Eklund⁵ and by Abrams, Kegeles and Hotte.⁶ The former used an initial acid precipitation followed by shaking with chloroform and salting out with ammonium sulfate, and the latter a combination of sodium sulfate and acid precipitation. Both preparations crystallized in the form of

² See the review by Eaton, *Bact. Rev.*, 1938, 2:3.

³ Eaton, *Jour. Bact.*, 1936, 31:347.

⁴ Pappenheimer, *Jour. Biol. Chem.*, 1937, 127:543.

⁵ Lamanna, McElroy and Eklund, *Science*, 1946, 103:613.

⁶ Abrams, Kegeles and Hotte, *Jour. Biol. Chem.*, 1946, 164:63.

Cannon and Sullivan (1932) recorded experiments which they regarded as indicating the local formation of antibodies at the site of an intradermal injection. Hartley (1940) injected a mixture of vaccinia virus and an aluminium hydroxide gel intradermally in rabbits. A nodule, in which the cellular reaction was predominantly macrophage, was produced at the site of inoculation. Extracts of the 4 day-old nodules contained from 2 to 10 times as much antibody as blood or other tissue extracts (see also Orskov and Andersen 1938, DeGara and Angevine 1943). Burnet (1941), on the other hand, could find no evidence of local antibody formation in the skin of rabbits by direct injection of a variety of soluble and particulate antigens. Oakley and his colleagues (1951), making local injections of diphtheria or tetanus toxoids into various tissues of rabbits and guinea-pigs in a state of secondary responsiveness to both these antigens, obtained good evidence of the formation, in fat and muscle and sometimes in skin, of antitoxin specific for the toxoid injected into the site.

Certain facts indicate, though they do not prove, the formation of antibody at or near the mucosal surfaces of the respiratory, alimentary and genito-urinary tracts. Thus Walsh and Cannon (1938) found, after intraperitoneal antigen, an even distribution of salmonella agglutinins in the nasal mucosa, lung and spleen, with a tissue : serum ratio of 1:10. After intranasal instillation of antigen, there was proportionately more antibody in the nasal mucosa. Moreover, when different antigens were given by the two routes, the proportion of antibody to the "intranasal" antigen in terms of serum antibody was higher in the nasal mucosa than in the spleen; and that of antibody to the "splenic" antigen was higher in the spleen than in the nasal mucosa.

Antibody occurs in the faeces. In guinea-pigs with experimental *V. cholerae* enteritis, antibody to the vibrio appears in the lumen of the gut before it is detectable in the serum (see Koshland 1953), and the varying ratios of antibody in serum and faeces of animals immunized with mixtures of different dysentery bacillus antigens (Barksdale and Ghosh 1951) is also consistent with a localized, and perhaps alimentary, origin of faecal antibody. Naylor and Caldwell (1953) record high urinary titres of agglutinins, not associated with high blood titres, in Egyptians with salmonella infections confined to the urinary bladder; and Kerr and Robertson (1953) found trichomonas agglutinins in uterine mucus, but not in the serum, of cows experimentally infected *in utero* with *T. fetus*.

The Rôle of the Reticulo-Endothelial System in the Formation of Antibodies.

We discussed in Chapter 17 the mechanism by which large particles like bacteria or red blood cells, or colloidal solutions of dye, are cleared from the blood stream after their intravenous injection and taken up in the phagocytic cells of the reticulo-endothelial system. Since many of these particles contain antigen, any consideration of the site of antibody formation must include the R.E. cells. It is economical of hypotheses to assume that the cells which capture the antigen also produce the antibody, and on that basis much of the earlier work was done.

Pfeiffer and Marx (1898) stated that in rabbits, which were undergoing immunization against cholera vibrios, bacterial antibodies might be present in the spleen, the bone marrow, and perhaps the lungs, in higher concentration than in the blood (see also Tsurumi and Kohda 1913, Cary 1922).

The removal, by splenectomy or inactivation, of the substantial amount of the reticulo-endothelial tissue present in the spleen affects antibody production. Deutsch (1899) recorded that splenectomy before the injection of typhoid bacilli into guinea-pigs had little effect on subsequent antibody production, but when carried out 3 to 5 days after the injection it led to a significant decrease. He also observed that, when the spleen was extirpated from a recently immunized guinea-pig and transferred to the peritoneum of a normal guinea-pig, anti-typhoid agglutinins appeared in the blood of the latter though to a relatively low titre. Similar results, both of diminished antibody production with

needles and were pure protein, having the properties of globulin and a molecular weight of about 1×10^6 . Type B botulinum toxin has been prepared as a pure homogeneous protein, though not crystallized, by Lamanna and Glassman.⁷ Tetanus toxin has been prepared in crystalline form by Pillemer, Wittler and Grossberg⁸ by methanol precipitation in the cold and similarly appears to be pure protein. These results definitely establish the protein nature of at least these bacterial exotoxins.

With the exception of botulinum toxin, the soluble toxins are destroyed by proteolytic enzymes; botulinum toxin is, therefore, the only toxin which is effective when given by mouth. There is no chemical evidence which explains, even in part, the toxicity of these substances; these proteins do not seem to differ in any essential respect from bland proteins, such as egg albumin. The amino acid composition of crystalline type A botulinum toxin, for example, is in no way unusual. It has been suspected that toxicity might be a property of a prosthetic group attached to the protein molecule, but this appears not to be true; the balance of evidence indicates that the toxicity is a property of the structure of the toxin molecule, possibly of the arrangement of the constituent amino acid molecules in the protein.

Evidence as to the mode of action of the bacterial exotoxins is, however, beginning to accumulate. The α toxin of *Clostridium welchii* is a lecithinase and the enzymatic activity accounts in large part for the hemolytic and other toxic properties of this substance. The Welch bacillus also produces a collagenase or κ toxin which, by attacking muscle collagen, may be at least partially responsible for the pulping of muscle seen in human gangrene. Pappenheimer⁹ has reported evidence which strongly suggests that diphtheria toxin may represent the protein moiety of an iron-containing respiratory enzyme, formed in abundance when the bacilli are grown in the presence of minimal amounts of iron; possibly its toxicity may be due to a competitive inhibition of respiratory enzymes of the host (p. 153). The mode of action of neurotoxins such as tetanus toxin is not yet understood.

The discovery that the toxic qualities of the soluble toxins are destroyed by treatment with formaldehyde which, at the same time, leaves the antigenic and antitoxin-combining properties of the toxin unimpaired, has been of the greatest practical importance in immunization procedures. Toxin so treated is called *toxoid* or *anatoxin*. It might be supposed that this observation would throw some light on the nature of the toxicity of these substances since formaldehyde is known to block amino groups—a fact taken advantage of in Sørensen's formal titration of amino acids. Analysis of the formaldehyde-protein derivatives indicates that the aldehyde combines with primary amino and primary amide groups, but not with secondary amide or phenolic groups. Because of the effect of formaldehyde on toxicity and the results of detoxification with ketene and phenyl isocyanate, some workers have supposed that the free amino groups of the toxin molecule, such as the ϵ -amino group of lysine, are intimately associated with toxicity, but the evidence for this is by no means complete.

⁷ Lamanna and Glassman *Jour. Bact.*, 1947, 54:575.

⁸ Pillemer, Wittler and Grossberg *Science*, 1946, 103:615.

⁹ Pappenheimer *Jour. Biol. Chem.*, 1947, 167:251.

Frankel and Grunenberg 1924, Ross 1926). Such discrepancies need cause no surprise. In a system of cells so widely distributed throughout the body, the effectiveness of any method of blockade must vary according to the experimental conditions. Blockade that falls short of completeness may, as some authors have suggested (Standenath 1923), stimulate rather than depress.

Other methods of injuring or disabling the cells of the reticulo-endothelial system have been used in attempts to study the part played by these cells in antibody production—the effect of X-irradiation (Benjamin and Sluka 1908, Hektoen 1915, 1918, 1920), of thorium X (Hektoen and Corper 1920), and of radium emanation (Hektoen and Corper 1922), of benzene (Rusk 1914, Simonds and Jones 1915, Hektoen 1916a), of toluene (Hektoen 1916b, Weaver *et al.* 1913), of dichloroethyl sulphide (mustard gas) (Hektoen and Corper 1921), and of trichloroethylamine (nitrogen mustard) (Philips *et al.* 1947). The results of these experiments, taken as a whole, lend support to the view that any procedure which severely injures the cells of the reticulo-endothelial system will substantially, and sometimes completely, inhibit the formation of antibodies. The time relation of the effect of X-rays is instructive. The results of Benjamin and Sluka (1908) indicated that depression of antibody response was less in animals irradiated four days before instead of immediately after the injection of antigen. Subsequent work (see, *e.g.*, Craddock and Lawrence 1948, Clemmensen and Andersen 1948, Kohn 1951, and the review of Taliaferro 1951) has in general confirmed the refractoriness of the immune response, once antibody formation has begun, to doses of X-rays that damage such components of the R.E. system as the bone marrow, lymphatic tissues and spleen. Taliaferro and his colleagues (1952) distinguished between a moderate general effect, perhaps due to a non-specific depression of protein synthesis, manifested by a retardation of antibody production, and a strong inhibitory effect induced by irradiation from 12 hours to 7 days after injection of antigen. There was no inhibition, however, when antigen was given either before or up to six hours after irradiation, suggesting that only the phase of uptake of antigen or its early katabolism by the tissues was radiation-sensitive. The phenomenon was also described by Dixon and his colleagues (1952) who deduced that, because the antibody formed in X-irradiated animals is indistinguishable from that in normal animals, the radiation-resistant cells are the site of antibody formation (Maurer *et al.* 1953).

These studies point to the participation of the R.E. system in antibody formation; but, owing to the radiation sensitivity of much of the lymphoid-macrophage system (see Chapter 47) to the doses of X-rays usually employed, they do not unequivocally indicate any one system.

There is little doubt that large-particle antigens are first taken up in the phagocytic macrophages of the R.E. system, and that the immediate fate of soluble antigens is similar; but in neither instance are these cells the sole depository of the antigen. Using a blue antigenic azo-protein in mice, Kruse and McMaster (1949) found the antigen in the cytoplasm of the R.E. cells, especially in cells of the liver and in the sinus and reticular cells of the lymph nodes. Coons and his colleagues devised an elegant immunochemical technique for the detection of antigen in cells by treating sections of tissue with specific antibody to which a fluorescent dye had been attached. On intravenous injection, both bacterial polysaccharide and serum protein antigens were found to be localized in the R.E. cells, where their concentration gradually increased, and in the parenchymatous cells of the liver, in the cells lining the renal tubules, in the capillary endothelium and in connective tissue cells. They were also found in low concentration in the lymphocytes and the lymphoid cells of the lymph nodes and spleen. Though the protein antigens disappeared within a few days and the polysaccharides after several months, they persisted longest and were most abundant in the R.E. cells of the liver, spleen and lymph nodes (Coons and Kaplan 1950, Kaplan *et al.* 1950, Hill *et al.* 1950, Coons *et al.* 1951).

In this connection we may note that Sabin (1939) attempted to correlate the disappearance of a coloured azo-dye protein antigen from the reticulo-endothelial cells of the rabbit with the antibody levels found in the serum. Her results suggested a connection between

The endotoxins are, in general, much more resistant to heat and to the proteolytic enzymes than are the soluble toxins. Many require heating to 80°–100° C. for one hour, and an endotoxin isolated from the meningococcus requires one hour at 120° C. for destruction; in contrast, the most resistant of the soluble toxins, botulinum toxin, is destroyed by exposure to 80° C. for ten minutes. Some of the endotoxins are destroyed by the proteolytic enzymes, but others appear to be immune to the action of these ferments. Similarly, formaldehyde has little or no effect on the majority of the endotoxins.

Some of the endotoxins may be protein, and it is thought by some that these substances are not toxic *per se* but are broken down in the animal body, either in the cells or body fluids, to toxic split products. The endotoxins of the enteric bacilli have been investigated in some detail and have been found to be polysaccharide-lipid-polypeptide complexes. The complex is broken down by mild acid hydrolysis and the toxicity disappears, though in some instances the lipid fraction may retain a small degree of toxicity. There is also some evidence that the endotoxin of the cholera vibrio is closely associated with a phospholipid fraction of the cell. Substances such as these would not, of course, be affected by formaldehyde or proteolytic enzymes.

Following the injection of exo- or endotoxins, a period of incubation elapses before symptoms of intoxication appear. This incubation period may be very short or may extend to thirty-six or forty-eight hours or longer. The usual incubation period of tetanus toxin is thirty-six hours but it may be reduced to thirty five to sixty minutes by the injection of very large amounts, 500,000 MLD, of crystalline toxin. It is sometimes stated that endotoxins have no incubation period, thereby differing from the exotoxins, but this is not generally true.

The pharmacological action of the soluble toxins resembles that of the vegetable alkaloids and is sometimes quite definite and characteristic for each toxin. Diphtheria toxin, for example, produces degeneration of the heart muscles, kidneys and liver and the hemorrhagic reaction in the adrenals which is a highly characteristic postmortem finding in the guinea pig. A part of tetanus toxin (tetanospasmin) has an affinity for the motor nerves. On the other hand, the symptoms and pathology resulting from administration of the endotoxins are not at all characteristic although minor distinctions have been reported. Animals inoculated with lethal doses of these substances usually show dyspnea, diarrhea and, in some cases, flaccid paralysis of the posterior extremities, become progressively weaker and finally die without having exhibited symptoms that might be regarded as characteristic of a particular endotoxin. The histopathology often indicates that the toxin exerts its effect on the blood vessels, damage to which results in degenerative changes in the tissue supplied. It has also been found¹⁰ that these substances affect the carbohydrate metabolism of the host, decreasing tissue glycogen, lactic acid and pyruvic acid, and specifically inhibiting succinic dehydrogenase, the relation of these changes to observed pathology is, however, not clear.

As has already been suggested, the bacterial toxins are not unique. Substances closely resembling the soluble toxins occur in the seeds of some of the higher plants and in the secretions of certain animals. Among the better

¹⁰ Kun and Müller. Proc. Soc. Exp. Biol. Med. 1948, 67,221.

The lymphocyte and antibody formation.—Ehrlich and Harris (1942) applied the double-antigen technique of McMaster and Hudack to the rabbit leg, with typhoid bacilli, sheep erythrocytes, and ovalbumin as antigens; in their hands the antibody maxima in efferent lymph and in the lymph nodes slightly preceded the maxima in germinal centres, whose increased numbers persisted some time after antibody levels were declining. The rise in antibody content of lymph from an immunized lymph node was accompanied by a rise in the cellular content. The cells, which were predominantly lymphocytes, contained substantially more antibody than the lymph and, since normal lymphocytes did not appear to absorb antibody, Harris and his colleagues (1945) suggested that the lymphocyte was the site of antibody formation. Similar results were obtained with two serologically distinct strains of influenza virus (Harris and Harris 1949b). Formation of antibody in the lymph nodes of the rabbit and horse was demonstrated by Oakley, Warrack and Batty (1949) after the local injection of secondary stimulating doses of diphtheria and tetanus toxoids; in horses, the nodes continued to produce antitoxin for several months. Habel and his colleagues (1949), on the other hand, found no experimental evidence of antibody formation in lymph nodes, or of transport of antibody by lymphocytes (but see Harris and Harris 1950).

The hypothesis of the lymphocyte as the site of antibody synthesis is in accord with the diminished antibody formation observed in animals whose lymphoid tissue as a whole is damaged or decreased by X-irradiation or nitrogen mustard (see, e.g., Schwab *et al.* 1950, Tahaferro and Tahaferro 1951) or by prolonged dosage with cortisone (Björneboe *et al.* 1951, Fischel *et al.* 1952, Malkiel and Hargis 1952); but as we noted above, this evidence does not point unequivocally to any one type of cell.

Small doses of X-rays induce a fall in circulating lymphocytes, and in immunized animals they are reported to increase serum antibody titres, presumably by lysis of cells ("lympholysis") and release of contained antibodies (Dougherty and White 1947; but see Roberts and White 1951); but others have failed to elicit this effect (Fischel *et al.* 1949, de Vries 1949; see also Marshall and White 1950). Dougherty and White recorded a transient rise in circulating sheep cell agglutinins in immunized rabbits, after a lympholytic dose of various adrenocortical hormone preparations, and postulated a hormone-induced release of antibody from these cells as part of the mechanism of antibody formation. The hormone effect was observed by others in immunized rabbits and rats (Eisen *et al.* 1947, Bussard *et al.* 1950, Hammon and Novak 1950), and amphibia (Bisset 1949). Bussard and his colleagues observed proliferative changes in the transitional and plasma cells of the spleen, as well as a lymphopænia, induced by the hormone. Fischel, LeMay and Kabat (1949) found no evidence of antibody release by adrenocorticotrophin (see also Havens *et al.* 1952). The view that antibody production is necessarily under hormonal regulation, or that the lymphocyte participates in regulation, either as a producer or transporter of antibody, remains to be established. In this connection we may note that, in immunized rats, the induction of lymphopænia by the administration of an anti-lymphocyte serum—a procedure presumed to destroy the lymphocytes—did not increase the circulating antibody (Woodruff *et al.* 1951); nor did the *in vivo* lysis of the lymphocytes by colchicine (Fagreu and Gormsen 1953). The presence of antibody in lymphocytes (see Harris and Harris 1950) is not consistent with the results of other experimental work (Craddock *et al.* 1949, Björneboe *et al.* 1947, Hammon and Novak 1950). Erslev (1951), for example, could find no antibody in the circulating lymphocytes of rabbits hyperimmunized with pneumococci, though the serum was rich in it. It will be realized that the evidence for the participation of the lymphocyte, as distinct from other lymphoid cells, in either the formation or the transport of antibody is both confused and contradictory. At present, the balance of the evidence is against the lymphocyte, at least as far as antibodies that can be detected by the orthodox *in vitro* techniques are concerned. The matter is, however, by no means settled. There is, moreover, a field of specific *in vitro* reactions involving antigen or hapten, but not detectable antibody, which are consistently associated with this type of cell. As we shall see in Chapter 51, certain forms of specific hypersensitivity

known toxins of plant origin (phytotoxins) are ricin (from the castor oil bean, *Ricinus communis*), abrin (from the jequirity bean, *Abrus precatorius*), and the similar substances, croton and robin. The more familiar examples of similar poisons of animal origin (zootoxins) include snake venoms, the poisons of scorpions and spiders and an actively poisonous substance present in eel blood. The chemical behavior and physiological action of these poisons are strikingly similar to those of the bacterial exotoxins. Antitoxins have been prepared against a number of these substances and the antivenins have been used with considerable success.

The "Invasiveness" of Bacteria. It was early suggested that, in addition to toxins, some of the highly virulent bacteria which invade the host tissues with great rapidity are enabled to do so by the secretion of soluble substances termed *aggressins* by Bail¹¹ and *virulins* by Rosenow.¹² Although subsequent experiment has provided evidence which strongly supports this general concept, these terms have, at present, little more than historical significance. It has been found that virulence in terms of tissue invasion and destruction of the defense mechanisms of the host by bacteria may be subdivided into a number of component factors. The more important of these are the *hemolysins* and *leucocidins* which destroy the red and white blood cells, the *coagulases* and *fibrinolysins* which influence the formation and dissolution of blood clots and a spreading factor which produces a marked increase in the permeability of the host tissues. Although some of these properties are associated with virulence, others are not so correlated and their relative importance is uncertain. It is probable that the ability of a bacterium to invade the tissues is not dependent upon any single property but rather upon a combination of properties, including both those that are known and others that are as yet unknown.

Hemolysins. A variety of bacteria produce hemolysins, substances which bring about the dissolution of the red blood cells of higher animals. The bacterial hemolysins, which are to be distinguished from the immune hemolysins formed by an animal in response to the injection of red cells of another species (p. 290), are of two types, the so-called filterable hemolysins which are extra-cellular and may be separated from the bacterial cells by filtration, and the hemolysins which are demonstrated by the cultivation of bacteria on semi-solid media containing whole blood.

The filterable hemolysins are sometimes named after the bacteria which form them, a *streptolysin*, for example, is a hemolysin produced by streptococci, and a *staphylolysin* a hemolysin of staphylococci. Hemolytic activity is demonstrated by the addition of filtrate or whole culture to a suspension of washed erythrocytes in physiological salt solution, after a period of incubation the red cells are laked and hemoglobin appears free in solution. The relation of these hemolysins to other naturally occurring hemolysins such as saponins, the hemolysins present in snake venoms and the like, is uncertain.¹³ The bacterial hemolysins appear to be proteins in nature, are inactivated by

¹¹ Bail Arch. Hyg., 1905, 52 272.

¹² Rosenow Jour. Inf. Dis., 1907, 4-285.

¹³ For a general discussion of hemolysis see the exhaustive monograph of Ponder *The Mammalian Red Cell and the Properties of Hemolytic Systems*. Protoplasma Monograph, No. 6, 1934.

Other cells.—There is no good evidence of liberation of antibody from polymorphonuclear leucocytes (see Fastier 1948, Walsh and Smith 1951). Girard and Murray (1954) took advantage of the increase in circulating monocytes produced by the lipid extractable from *Listeria monocytogenes* to investigate the rôle of these cells. When a high monocyte count was maintained in rabbits by this means, antibody response to the typhoid bacillus and to staphylococcus toxoid was increased. Moreover, when a predominantly monocyte pleural exudation was induced in such animals during the secondary antibody response, the cells contained more antibody than the fluid. This was not, however, evidence of antibody formation by the cells, because passively administered antibody proved to be concentrated within them.

As regards other tissues, it is noteworthy that muscle does not appear to synthesize antibody (Keppie and Macfarlane 1948). The liver, though turning out most of the plasma proteins, does not in the rat appear to synthesize globulin (Miller and Bale 1954); if this proves to be generally true, then we may exclude the Kupffer cells of the R. E. system as a major source of antibody.

The Formation of Antibodies in Tissue Cultures.—A method which offers obvious possibilities for the study of antibody formation is that of tissue culture.

Carrel and Ingebrigsten (1912) cultivated fragments from the bone marrow and lymph glands of guinea-pigs in the homologous blood plasma, and added to the cultures small amounts of red cells from the goat, against which the serum of the guinea-pig showed no lytic action. Hæmopsonins appeared in the culture on the 3rd day, as judged by direct observation of the degree of phagocytosis. Hæmolysins were detectable on the 4th day, and had increased markedly in activity on the 5th. The hæmolysins could be specifically absorbed from the fluid by the usual technique. Lüdke (1912) injected killed cultures of typhoid or dysentery bacilli into rabbits, removed fragments of the spleen or bone-marrow after 1 to 5 days, and cultivated them in homologous plasma. After 2 to 5 days' incubation he was able to detect lysins and agglutinins in the culture fluid. In a similar way he succeeded in producing hæmolysin for ox or sheep corpuscles. His results were for the most part negative when he added the antigenic materials directly to the tissue cultures. Przygode (1913, 1914), Reiter (1913) and Schulf (1926) also recorded positive results. Subsequent workers, including those we have mentioned above in the section on antibody production by plasma cells, have demonstrated antibody formation against a variety of antigens when the cultures were made from the tissues of already inoculated animals; but the results of adding the antigen after the establishment of the tissue culture *in vitro* have been uniformly negative (Meyer and Lowenthal 1928, Hwon 1937, Parker 1937, Salle and McOmie 1937, Beard and Rous 1938). The reasons for the failure of *in vitro* immunization are not apparent, though Beard and Rous, who used a pure culture of Kupffer cells from the liver, note that the cultivated cells had lost their normal *in vivo* power of ingesting antigenic particles.

Conclusions with regard to the Site of Formation of Antibodies.—Taking the evidence as a whole the conclusions to be drawn do not seem to be in doubt in spite of various discrepancies. There is ample evidence that the lymphoid element of the lymphoid-macrophage system (Chapter 47) is concerned with antibody formation. The researches of the past decade clearly indicate that an adaptive cellular response occurs *pari passu* with the antibody response, in organs which, like the spleen, are usually credited with antibody formation, and in lymph nodes, and in discrete regions of connective tissue stimulated by antigen. The site of this response varies with the route of injection of the antigen, and it is possible that the type of cell produced varies significantly with the antigenic material. The evidence implicating any particular kind of cell as the site of antibody synthesis is largely circumstantial, and although refinements of technique have led to a

heating (55° C. for thirty minutes), and are antigenic, *i.e.*, when injected into animals they stimulate the formation of antihemolysins.

That hemolytic activity is a property of a group of substances of bacterial origin rather than of a single substance formed by a number of bacterial species is indicated not only by differences in immunological specificity but also by the varied properties of the activity. Many hemolysins, for example, are oxygen-stable, but some, produced by the pneumococcus and certain strains of streptococci, are oxygen sensitive, *i.e.*, they are active in the reduced form but inactive in the oxidized form, the oxidation-reduction being reversible at low temperatures.¹⁴ Hemolysins further differ from one another in their heat and acid resistance and in the incubation time which precedes visible laking of the red cells. Differences in their activity on the erythrocytes of various species of higher animals may be marked, a given hemolysin, for instance, may

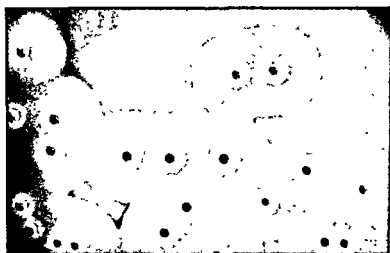


Fig. 28. "Hot-cold" hemolysis. *Staphylococcus aureus* on sheep blood agar. Produced by holding the plate alternately at incubator and refrigerator temperature. The small clear zones are produced in the incubator by the α lysin and the large zones in the refrigerator by the β lysin.

lyse sheep cells but not rabbit cells. In general the cells of the animal species from which a bacterium is isolated are more sensitive to its hemolysins than are the cells of other animal species.

A single bacterial strain may form more than one hemolysin. Certain strains of staphylococci form at least two hemolysins, one acting on both α and β and the other β only.¹⁵

The former is termed α lysin and the latter β lysin. The action of both can be shown in culture on sheep blood agar, the α lysin producing a zone of complete clearing on twenty four hours' incubation and the β lysin producing a zone of darkening which becomes lighter and clear on subsequent refrigeration of the plate.¹⁶ This phenomenon is illustrated in Fig. 28

¹⁴ Cf. Todd Jour. Path. and Bact., 1934, 39 299.

¹⁵ Glenn and Stevens Jour. Path. and Bact., 1935, 40 201.

¹⁶ Christie and Graydon. Australian Jour. Exp. Biol. & Med. Sci., 1941, 199.

The minute dose of 10,000 bacilli per kilo. body weight, administered to rabbits, results in no detectable agglutinins. With ten times the dose we pass the threshold and obtain an average titre of 1/330. Over the range 10^5 to 10^8 bacilli a thousandfold increase in dose results in about a tenfold increase in titre—the critical student glancing at the numbers in the second column will not place too much reliance on the mean values recorded in the third. When we increase our dose still further we shall get still higher titres; but we shall be reaching the point where our inoculum attains an appreciable toxicity, and we shall, apart from this, be subject to the law of diminishing returns. However high we push our dose, up to the limit that will kill our rabbit, we are unlikely, after any single inoculation, to reach a titre of more than 1/10,000–1/20,000.

In general, there is a minimal threshold value, below which no response is obtained. Above this value the titre attained varies with the dose administered, but in such a way that the increase in titre is relatively much smaller than the increase in dose required to produce it. Eventually a point is reached at which further increase in dose results in little or no increase in antibody production.

TABLE 80

SHOWING THE MEAN VALUES OF THE HIGHEST TITRE OF FLAGELLAR AGGLUTININS IN SMALL GROUPS OF RABBITS INJECTED INTRAVENOUSLY WITH DIFFERENT DOSES (PER K.B.W.) OF *Salm. paratyphi B*.

No of Bacilli injected per k b w.	No. of Rabbits tested.	Highest Titre. (Mean Value).
10^3	3	3,540
10^7	3	1,860
10^8	6	330
10^4	4	0 ¹

¹ = no agglutination at a dilution of 1/4.

Not all antigens induce this kind of dosage response. For example, even very small doses of the acetyl polysaccharide on the Type I pneumococcus call forth the production of antibodies (Avery and Goebel 1933, Enders and Wu 1934). Downie (1937) found that an effective immunizing dose in mice lay between 1.0 and 0.01 μg .; larger doses, of 100 or 1,000 μg ., proved to be ineffective.

Immunological paralysis—Massive doses of antigen may completely paralyse the response of the antibody-forming apparatus. We discussed in Chapter 49 Felton's demonstration of such an immunological paralysis in mice given an overwhelming dose of pneumococcal polysaccharide—enough to remain for a long time detectable in the tissues. The analogous phenomenon, of specific induced tolerance, is elicited by the introduction of antigen into the foetal mouse or chick before its capacity to synthesize antibody has matured. With maturation of this capacity after birth, the animal is indifferent to stimuli by the antigen employed (Billingham *et al.* 1953).

Variations in Response to Different Antigens.—Fig. 253 shows the effect of injecting into an animal (a dog) a particular antigen (rat corpuscles) against which it possesses a normal antibody (haemagglutinin). The immediate effect of the injection is to cause a fall in the normal antibody titre, followed by a rapid rise of the type depicted in Fig. 252. This *negative phase* is a characteristic result of injecting any large amount of antigen into an animal that possesses the corresponding antibody in its circulating blood.

Similarly, some strains of streptococci produce two hemolysins, one of which is relatively heat-stable but oxygen-sensitive and the other oxygen-stable but heat-sensitive.¹⁴ It has been shown by Bernheimer¹⁷ that streptolysin O, the oxygen-labile hemolysin, has a toxic action on the isolated frog heart. This cardiotoxic action is of particular interest in that it appears only on second application following a first or sensitizing application which releases an inhibitor and leaves the tissue susceptible to the action of the cardiotoxin.

The production of hemolysins is favored by inclusion of serum in the broth in which the microorganisms are cultured, and it is of some interest that the activity of the hemolysin formed in such media is most marked on the red cells of the animal species from which the serum was derived.¹⁸ The hemolytic activity of a culture may be transitory, probably because of inactivation of formed hemolysin. Some hemolysins lose their activity when incubated at 37° C. for two hours or more; for example, de Kruif and Ireland¹⁴ found a maximum hemolytic titer after eight hours' incubation of streptococcus cultures which declined rapidly thereafter so that in many cases no activity could be detected in filtrates of fourteen-hour cultures.

Blood-Plate Hemolysis. The colonies of some bacterial species on blood agar produce visible changes in the medium immediately surrounding the colony which are termed "hemolysis." Two general types of change are observed, one designated as α or green hemolysis, in which the bacterial colony is surrounded by a zone of greenish discoloration, and the other, β -hemolysis, in which the zone around the colony is clear and uncolored, in contrast with the red opacity of the medium. According to Brown¹⁹ the zone of discoloration of α -hemolysis is surrounded by a very narrow clear zone, but this is not apparent except upon careful examination. Microscopic examination of the green zone shows the presence of many discolored corpuscles, but in β -hemolytic zones corpuscles cannot be found.

The relation between blood-plate hemolysis and the filterable hemolysins is usually regarded as uncertain, for, although microorganisms hemolytic on blood plates frequently do not appear to produce filterable hemolysin, some workers maintain that, since filterable hemolysins are difficult to demonstrate, failure to find them is not significant. It would appear that the processes involved are different, in the case of the filterable hemolysins the permeability of the red cell is altered and the hemoglobin escapes into the surrounding fluid, while in blood-plate hemolysis the pigment is broken down to green or colorless compounds. In the case of the cholera vibrio the two processes have been sharply differentiated,²⁰ since the identification of this organism is dependent in part on the hemolysis of goat erythrocytes in suspension (Grieg test), though the true cholera vibrio is negative to this test, some strains show hemolysis on blood agar plates. Van Loghem has distinguished between the hemolysis of goat erythrocyte suspensions and what he has termed hemodigestion on blood agar. On the other hand, there appears to be a close association between β -hemolysis and streptolysin O. Green hemolysis was formerly thought

¹⁴ Bernheimer and Cantoni: *Jour. Exp. Med.*, 1945, 81:295, 307.

¹⁸ de Kruif and Ireland. *Jour. Inf. Dis.*, 1920, 26:285

¹⁹ Brown: Rockefeller Institute for Medical Research, Monograph No. 9, 1919.

²⁰ Especially by van Loghem see *Centralbl. f. Bakt., I Abt. Orig.*, 1926, 100:19.

and Hektoen and Boor (1931), on the other hand, have recorded results which indicate a far less restricted range of activity. The latter observers immunized rabbits

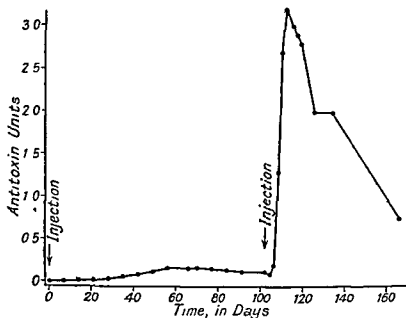


FIG. 255.

Antitoxin production in the horse: showing difference between primary and secondary response. (After Glenny and Südmersen.)

and Waddington 1925, Glenny and Waddington 1926, 1928) makes it clear that the simultaneous injection of several antigens may considerably reduce the titre of antitoxin obtained. It seems probable, as Glenny suggests, that this "crowding-out" effect is particularly prone to occur when any one antigen is present in great excess. In guinea-pigs inoculated with tetanus toxoid mixed with a second antigen—either diphtheria toxoid or a typhoid vaccine—Barr and Llewellyn-Jones (1953) demonstrated a crowding-out of the tetanus antitoxin response when the animals already had a basic immunity to the second antigen, as a result of which the second antigen induced a substantial secondary response that apparently occupied most of the animals' capacity to synthesize antibody (see also Barr and Glenny 1952).

Bjorneboe (1941, 1943, 1945) immunized rabbits with vaccines containing from one to eight types of pneumococcus, which were given every second day for 8 months; the antibody production per type was the same for mixtures up to three types, and became progressively weaker as the number of antigens increased. When the "ceiling" values of serum antibody were attained, all the serum globulin appeared to consist of antibody. With hyperimmunization the globulins might rise to over seven times the normal value; this was accompanied by an apparently compensating decrease in serum albumin. The separate antitoxic response of rabbits to mixtures of diphtheria, tetanus and staphylococcal toxoids (Ramon and Richou 1940) and of diphtheria and staphylococcal toxoids given intranasally (Sato and Kodama 1940) was found to be similar to that following injections of the single antigens.

In man, a combination of two or more antigens is reported by some to have a synergic effect, each antigen being more effective in combination than alone. For example, synergic effects are recorded for mixtures of diphtheria and tetanus toxoids, with and without typhoid vaccines (Sacquepée, Pilod and Jude 1936a, b); for staphylococcal and diphtheria toxoids (Kodama, Sato and Hata 1940); for tetanus toxoid and typhoid vaccines (Ramon 1936, Maclean and Holt 1940; see also Grasset and Girdwood 1940, Fraser *et al.* 1943); for diphtheria and tetanus toxoids (Bigler and Werner 1941), and for these two toxoids

by repeated intravenous injections of mixtures containing large numbers of different antigens, each in a relatively pure state. These included hæmoglobin from the ox, cat, dog, hog, horse, man, sheep and turkey, horse pseudoglobulin, serum albumin from chicken and man, egg albumin and other constituents. One of the mixtures contained 14 different antigenic constituents, the other 35. Precipitins were produced against almost all the antigens injected, and in most cases to high titre.

In the case of antitoxins the careful work of Glenny and his colleagues (Glenny 1925a, Glenny, Hopkins

to be due to the formation of methemoglobin and associated with the formation of hydrogen peroxide, but it has been found²¹ that the green substance is an iron-containing derivative of hemoglobin very possibly formed by reduction. The mechanism of β -hemolysis is not well known, but in the case of staphylococcal hemolysins, the hemoglobin is not destroyed; according to Christie and Graydon¹⁶ the clearing around the colony is due to migration of the liberated hemoglobin. As in the case of the filterable hemolysins, hemolysis on blood agar is frequently species-specific; a higher proportion of bacterial strains will show hemolysis on blood from one animal species and not on that of another.

The relation of ability to form hemolysin and virulence is obscure. Although among some of the parasitic bacteria such as streptococci and staphylococci, virulence is associated with hemolytic activity, a number of saprophytic bacteria also produce hemolysins. On the other hand, it has been reported that in staphylococcus infections of the udder, the hemolytic strains are much more irritating than the non-hemolytic organisms. It might be expected that infection with actively hemolytic bacteria would be accompanied by anemia and hemoglobinuria but this is not the case, as a rule; infections with *Clostridium welchii*, however, are frequently characterized by a gross blood destruction and consequent severe anemia and jaundice which is due to the continuous release of the hemolytic α -toxin (p. 581). It is also not unlikely that hemolysins may exhibit toxicities other than that of the lysis of erythrocytes, as, for example, in the case of the cardiotoxic action of streptolysin O noted above.

Leucocidins. A number of bacteria, notably staphylococci, streptococci and pneumococci, form leucocidins, substances which kill and, in some cases, lyse, polymorphonuclear leucocytes. The death of the leucocytes results in the inability of these cells to reduce methylene blue, a phenomenon made use of by Neisser and Wechsberg²² and others to demonstrate the activity of these substances. A direct microscopic method has been introduced²³ which has shed some light on the effect of these substances on the white blood cell. Human and rabbit leucocytes in contact with leucocidin become spherical, the nuclei fragment and the cells may, with some leucocidins, eventually burst or, with others, remain intact.

The amount of leucocidin produced by one bacterial species may vary widely from one strain to another, and a single strain may produce more than one leucocidin. The detection of more than one leucocidin is dependent upon differences in thermostability and in the kind of leucocytes attacked, one staphylococcal leucocidin, for example, affects both rabbit and human leucocytes, while another is active only on rabbit cells. In general, the leucocidins closely resemble the filterable hemolysins in that they are antigenic, variable in their heat resistance, etc., and, in some cases, may be identical with hemolysins. The α -hemolysin of staphylococcus, for example, is a leucocidin.²⁴ They may not be strain specific; some of the staphylococcal leucocidins, for example,

²¹ Cf. Anderson and Hart: Jour. Path. and Bact., 1934, 39:465.

²² Neisser and Wechsberg: Ztschr. Hyg. u. Infektionskr., 1901, 36:299.

²³ Valentine, Lancet, 1936, 1:526. It should be pointed out that it is claimed by some workers that the leucocidin tested by one method is not the same as that tested by the other.

²⁴ Wright: Lancet, 1936, 1:1002.

antigenic material is not limited to the production of diphtheria antitoxin, though it is particularly well marked in this case. Fig. 256 shows a similar response to an injection of horse serum in a rabbit which had previously been injected with that antigen, but whose precipitin titre had since sunk to zero.

The same holds true, in regard to the comparative titre attained, though not in regard to the prolonged induction period after the primary stimulus with large-particle antigens, such as bacteria. Table 81 sets out the primary and secondary response of rabbits to the intravenous injection of 10^5 *Salmonella paratyphi B* per kilo. body weight. It will be noted that the titres given are not the maxima attained but the average titre during the 50 days after inoculation. This value was selected because the curve of agglutinin titre after a secondary inoculation tends not only to rise higher than after a primary injection, but to remain high over a longer period.

These three are examples with protein antigens. Not all antigens stimulate a secondary response. Burnet and Freeman (1938), for example, could induce no secondary rise of antibody in rabbits primarily and secondarily stimulated by intravenous injection of Q fever rickettsiae. Heidelberger and his colleagues immunized man with the type-specific pneumococcal polysaccharides. After a

TABLE 81

SHOWING THE AVERAGE TITRE OVER A PERIOD OF 50 DAYS AFTER PRIMARY AND SECONDARY INJECTIONS INTO RABBITS OF 10^5 *Salmonella paratyphi B* PER K.B.W.

Rabbit.	Primary Average Titre.	Interval between 1st and 2nd Injections.	Secondary Average Titre
R11	280	147 days	2,720
R19	37	147 "	1,720
R25	130	147 "	430
R30	130	133 "	1,230
R31	91	133 "	1,230
R33	110	133 "	2,010

small primary inoculum the serum content of specific antibodies rose slowly for several weeks, remained high for 5 to 8 months, and dropped to 20-50 per cent. of this maximum value in the next 3-6 years. A secondary inoculum had little effect after eight years, except in a few subjects with a low titre of antibody, and even here the effect was slight (Heidelberger *et al.* 1946, 1950; but see Murray *et al.* 1950).

The difference between the primary and secondary response is sufficiently obvious and needs no comment. With very small doses there may be no detectable primary response, antibodies failing to appear till after the second or later injection of a bacillary suspension, as illustrated in Table 82.

Burnet (1941) has emphasized a distinguishing feature of the secondary response that is especially obvious when the stimulating dose of antigen is given intravenously. In the secondary response to staphylococcal toxoids, herpes virus and a bacteriophage, after a delay of 1 to 2 days, the antibody content of the serum increased exponentially with regard to time, until the maximum level was approached, when the rate of increase in antibody content gradually diminished to a low value. In Fig. 257, for example, where the staphylococcal antitoxin content of the serum is plotted logarithmically, the curve is practically linear from the 2nd to the 4th day (see also Holt 1951).

are immunologically similar if not identical regardless of the bacterial strain from which they are derived.²⁵

The part played by the leucocidins in the virulence of a bacterium is obscure. Theoretically, it should be of some advantage to an invading microorganism to be able to destroy these phagocytic cells and thereby break down, to some degree, the defenses of the body. The polymorphonuclear leucocytes (heterophils) are not, however, the most important cells in the phagocytic destruction of bacteria (p. 294), and the effect of these substances on the other phagocytic cells, the mononuclears, the histiocytes and others is not known. As in the case of the hemolysins, the leucocidins of bacterial origin must be differentiated from the immune leucocidins, those produced by the animal body in response to the injection of leucocytes from another species.

Coagulase²⁶ and Fibrinolysin. The formation of blood clots is accelerated by a substance, coagulase, formed by some bacteria, and the clots formed, either with or without the acceleration of this substance, may be dissolved by other substances of bacterial origin, the fibrinolysins. The production of coagulase, is, so far as present information is concerned, confined to the staphylococci for the most part, and may be causally related to the thrombi that are common in infections with these organisms. Occasional strains of other organisms such as *Pseudomonas pyocyaneus*, *Bacterium prodigiosum*, *Bacterium coli* and *Bacillus subtilis* have, however, been observed to accelerate the clotting of blood. Coagulase activity is demonstrated by the addition of bacteria to citrated or oxalated blood plasma (usually rabbit), which gels within three hours. Cell-free coagulase has, however, been prepared from plasma cultures of *Staphylococcus aureus*.²⁷ Coagulase is regarded by some as a precursor of a thrombin-like substance and requiring an activator present in some, though not all, plasmas.²⁸ Unlike coagulase, the thrombin-like substance is thermolabile. The clotting produced by coagulase is not, however, inhibited by "antithrombins" such as heparin or hirudin. The activity is not filterable and is relatively thermostable; it is only partially destroyed by exposure to 100° C. for thirty minutes. Other properties such as precipitation with alcohol and acid and ammonium sulfate and inactivation with trypsin and pepsin suggest a protein nature. Its antigenicity is uncertain. The relationship of coagulase to virulence is obscure, although it is often said that there is no association between the two. It would seem that the property of accelerating the clotting of blood might, on the one hand, be unfavorable to further invasion of the host tissues by a microorganism forming this substance, and, on the other, serve as a temporary protection against the defenses of the host. Hale and Smith²⁹ have observed that coagulase-producing staphylococci are less susceptible to phagocytosis in the presence of plasma than are coagulase-negative strains, presumably a result of coating the bacteria with a film of coagulum.

²⁵ For a general study of these substances see Todd Brit. Jour. Exp. Path., 1942, 23: 136.

²⁶ For a summary of information regarding coagulase see the review by Blair Bact. Rev., 1939, 3:97

²⁷ Lominski Nature, 1944, 154 640.

²⁸ Smith and Hale. Brit Jour Exp Path., 1944, 25:101.

²⁹ Hale and Smith Brit. Jour. Exp. Path., 1945, 26 209; *ibid.*, 1947, 28:57.

It is probable that secondary responsiveness lasts for some time after serum antibody is no longer detectable. Murray, Ofstrock and Snyder (1952) found that secondary responsiveness to typhus vaccine in man might last for 5 to 6 years.

TABLE 82

SHOWING THE HIGHEST TITRE ATTAINED AFTER 1ST, 2ND AND 3RD INJECTIONS OF 10^4 *Salm. paratyphi B* PER K.B.W.

Rabbit.	Highest Titre after 1st Injection	Interval between 1st and 2nd Injections.	Highest Titre after 2nd Injection.	Interval between 2nd and 3rd Injections	Highest Titre after 3rd Injection.
R16 . . .	0	28 days	8	92 days	40
R32 . . .	0	28 "	32	92 "	319
R46 . . .	0	28 "	7	92 "	20
R47 . . .	0	28 "	0	92 "	0

It must not be supposed that the differences between a primary and secondary response are always so clear-cut as those depicted here. The curves may, however, be regarded as giving a true picture of the kind of differences observed. It will be noted in Figs. 258 and 259 that the primary response may be quite brisk and result

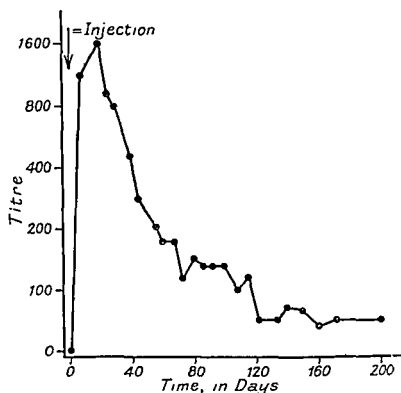


FIG. 259.

Agglutinin production: response of an uninoculated person to a single injection of typhoid vaccine.

level, which varies widely with the nature of the antigen employed and the responsiveness of the animal injected, it becomes impossible to induce any further rise in the concentration of antibody in the circulating blood.

Fig 261 (p 1275) shows the effect of repeated subcutaneous injections of typhoid bacilli in a rabbit (Goldberg 1901). Each successive injection raises the agglutinin

in a high titre of agglutinins. This, for instance, is the usual experience as regards the production of H agglutinins in response to the injection of an optimal dose of flagellated bacilli.

We have seen that increases in the dose of antigen administered at a single injection are subject to the law of diminishing returns: in just the same way the repetition of injections, beyond a certain number, is usually found to produce less and less effect with each successive dose. When the titre of a particular antibody has been forced up to a certain

Fibrinolysin.³⁰ Perhaps more important to the virulence of a bacterium is the ability to dissolve formed blood clots through the agency of fibrinolysin. It has been observed that if a small amount of bacterial culture is mixed with oxalated plasma and the latter allowed to clot by the addition of calcium chloride, the fibrin clot may be dissolved within a short time as a result of bacterial activity. The activity may be titrated by determining the highest dilution that will lyse a fibrin clot in one hour at 37° C. At present it appears that the ability to bring about this lysis is confined to certain groups of streptococci, although other bacteria such as staphylococci, the plague bacillus and others have been described as fibrinolytic. Like the hemolysins, the fibrinolysins appear to exhibit some degree of specificity with respect to the source of the fibrin, streptococci isolated from human infections, for example, will lyse clotted human plasma more rapidly than plasma clots from other animals. Fibrinolysin appears to fall into the same general category as the toxins, hemolysins and the like in that it is found in culture fluid freed from bacterial cells, appears to be protein in nature, and is antigenic. Fibrinolysin is, however, distinct from these other substances; although a high correlation has been observed between the occurrence of filterable hemolysin and fibrinolysin, in many cases a bacterial strain is able to bring about the dissolution of erythrocytes but not solution of clots, and *vice versa*.

The nature of the lytic process has been of some interest, and it has been found³¹ that fibrinolysin is an activator of an inactive proteolytic enzyme or "lysin factor" present in the serum. Thus the term fibrinolysin is a misnomer, and possibly the active enzyme should be termed "plasmin." In any case, the lytic process is accompanied by an increase in amino nitrogen and other manifestations of proteolysis.

Fibrinolysin is antigenic and fibrinolysins from different strains of bacteria appear to be very similar if not identical immunologically. Antisera specifically inhibit the activity and the occurrence of anti-fibrinolysin in the serum has been useful as an indicator of past infection with fibrinolysin-positive streptococci.

It should be noted here that a number of bacteria exhibit a tendency to inhibit the clotting of plasma, a property that may be demonstrated by delaying the addition of calcium to the oxalated plasma; when calcium is added after a period of preliminary incubation, the clot fails to form. This property has been most often demonstrated with cultures in dextrose broth, and in some cases, at least, the failure of the plasma to clot is a result of the presence of lactic and other acids formed in the fermentation.

Fibrinolysin appears to be closely associated with virulence and particularly with the ability to invade the body tissues. Since one of the first reactions of the body to tissue destruction is the formation of blood clots which tend to wall off and isolate the infected region, it is not surprising that a bacterium capable of lysing these clots should show marked tendencies to extensive tissue invasion. The streptococci are among the most invasive of the pathogenic forms, and this

³⁰ See the review by Tillet. *Bact. Rev.*, 1938, 2 161.

³¹ See Christensen. *Jour. Gen. Physiol.*, 1945, 28:363, 559, Holmberg. *Arkiv Kemi Mineral. Geol.*, 1944, 17A 8.

of adjuvant substances to the antigen. With regard to the route of immunization, it is generally held that antigens in the form of large particles, like bacteria or animal erythrocytes, are most effective when given intravenously (see, for example, Marinelli 1937), whereas soluble antigens are best given into the tissues. If a soluble antigen is attached to coarsely particulate matter, it may be most effective by the intravenous route (Freund and Bonanto 1941). A probable cause of the relative inefficacy of intravenously injected soluble antigens is their rapid elimination from the body. The same factor probably diminishes the stimulus of antigens injected into the tissues, for most of the procedures that increase antigenicity appear to act by hindering the release of antigen from the injection site.

Variation in the volume of the inoculum.—The experiments of Hartley (1935) indicate that the total volume of an immunizing injection, as well as its content in antigenic material, has a significant influence on the antibody response. A series of guinea-pigs were injected with a constant dose of diphtheria toxoid made up in a total volume of 0.5 ml. or 5.0 ml. of saline. In repeated tests it was found that the animals receiving the larger volume gave a far better immunizing response. Timmerman and Brandwijk (1936*a, b*) confirmed Hartley's results. Schmitz (1938), however, repeated the test in medical students, and found no difference either in antitoxin response or in the rate at which antitoxin disappeared from the blood.

Dispersal of the inoculum through the tissues.—A stimulation of response resembling that of Hartley (1935) may be obtained by subdividing a dose of antigen into several smaller doses and injecting each into different sites in the animal. The response is greater than that produced by the whole dose administered singly (Ramon, Lemétayer *et al.* 1937, Miles and Pirie 1939).

The addition of adjuvants.—Adjuvants are usually intended for injection into the tissues, and are for the most part mildly irritant substances. Alum is one of the most widely used adjuvants. It was introduced by Glenny and his colleagues (Glenny *et al.* 1926) for improving the antigenicity of diphtheria toxoid, but has since been applied to other toxoids and to bacterial vaccines like those of *H. pertussis* (Chapter 74). Aluminium hydroxide is also effective (Hektoen and Welker 1933). Other successful adjuvants are lanoline in oil, cholesterol, salts of calcium and magnesium (Ramon *et al.* 1935); tapioca (Ramon 1937, 1939, Schmidt and Steenberg 1936); bacterial products like typhoid vaccine (Ramon 1936), staphylococcal toxin (Burky 1934, Swift and Schultz 1936); tubercle bacilli (Freund and McDermott 1942); serum containing heterophile antibodies (Kalinin *et al.* 1935). Both Glenny and Ramon attribute the action of alum and tapioca respectively to the stimulation of tissue lesions, from which the antigen is slowly liberated, thus providing a prolonged and continuous antigenic stimulus. We noted in the previous section that *H. pertussis* itself may have an adjuvant action. *Br. abortus* is also effective (Ramon *et al.* 1950).

The lesion produced by a single injection of alum-precipitated diphtheria toxoid is apparently the chief source of antigen for some time after its formation. Thus Blagowechensky (1938) excised the lesion made by alum-toxoid at varying intervals after its formation in the skin of guinea-pigs and found that, within a period of 10–20 days, the degree of immunity reached depended almost entirely on the length of time the lesion had remained in the body.

The adjuvant effect has also been attributed to the high absorptive capacity of the substances employed. Aluminium hydroxide, for example, will absorb proteins *in vitro* (Hektoen and Welker 1933, Cohen and Mosko 1943, Holford, Ludden and Stevens 1943). Faragó and Ujhelyi (1942) injected groups of guinea-pigs with diphtheria toxoid alone, with toxoid and $Al(OH)_3$ in the same injection sites, and with toxoid and $Al(OH)_3$ in different sites. The average antitoxin responses in unit per ml. after four weeks were 0.005, 0.4 and 0.02 respectively. In the last group the toxoid was apparently absorbed

phase of their virulence may be attributed in part to the formation of fibrinolysin.

Hyaluronidase (Spreading Factor, Invasin).³² It has been found in recent years that the permeability of the tissues is remarkably increased by a factor, often called the *Duran-Reynals factor* after its discoverer, present in certain mammalian tissues, notably the testes. Bacteria, vaccinia virus, and substances such as toxins, india ink and the like, diffuse rapidly from the site of inoculation when injected in conjunction with extracts containing this factor. Strains of staphylococci and streptococci that have no pronounced invasive powers may, in this way, be rendered highly invasive. This factor is also present in a number of bacteria notable for their invasive properties, such as certain strains of staphylococci and streptococci, pneumococci and certain of the obligate anaerobes such as the bacillus of gas gangrene. Among some of these organisms there is some degree of association between content of the Duran-Reynals factor and virulence; the non-invasive strains of staphylococci noted above contain little or none of this factor but become invasive when it is supplied, while the invasive strains produce the factor themselves. This substance has been found to be an enzyme, hyaluronidase, whose substrate is hyaluronic acid, a mucopolysaccharide consisting of acetyl glucosamine and glucuronic acid, which acts as a cement substance of the tissues and is found in synovial fluid and elsewhere in the body. The action of the enzyme in decreasing the viscosity of hyaluronic acid by hydrolysis is responsible for facilitating the penetration of the tissues by bacteria that produce it. The effect on the tissues is temporary in that, following inoculation of preparations of the enzyme, the dermal barrier is restored partially in twenty-four hours and completely in forty-eight hours.³³ It is of some interest that hyaluronic acid is also found in the capsular substance of some strains of streptococci. Such strains do not produce hyaluronidase and in its presence are denuded of capsules and more readily phagocytosed (see below). The ability of such strains to invade the tissues does not, of course, depend upon their elaboration of hyaluronidase. The activity appears to be antigenic, in that it is neutralized by antisera, but immunologically distinct from different sources; it has been suggested that the enzyme is combined with different proteins in different organisms.

Hyaluronidase is antagonized by an enzyme present in normal blood plasma which has been called anti-invasin I. An enzyme in bacteria, pro-invasin I, destroys anti-invasin I, and another enzyme in plasma, anti-invasin II, destroys pro-invasin I. It has been suggested that a balance between all these in the host-bacterial system determines whether or not invasion of the tissues will occur.³⁴

Capsules. The remarkable association between the presence of a capsule on a pathogenic bacterium and its virulence has been discussed elsewhere (Chapter 6). The capsular material, generally polysaccharide in nature al-

³² Cf. the reviews by Duran-Reynals: *Bact. Rev.*, 1942, 6:197, and by Meyer. *Physiol. Rev.*, 1947, 27:335.

³³ Hechter *Proc. Soc. Exp. Biol. Med.* 1948, 67:343

³⁴ Haas *Jour Biol. Chem.*, 1946, 163:63, 89, 101.

antigens were given without adjuvant. In the granulomata, the antigens were detectable in macrophages; and these were devoid of antibody.

It is generally believed that many adjuvants act by diminishing the rate at which antigen is released from the site of inoculation, or from cells which have taken it up. With Freund's adjuvant, Herdegen, Halbert and Mudd (1917) recorded the persistence of *Sh. flexneri* antigen at the injection site in mice for over 20 days, and Talmage and Dixon (1953) detected a large amount of serum globulin at a subcutaneous site 20-30 days after an inoculum which, given without adjuvant, largely disappeared within 5-10 days. The detoxifying action of many adjuvants is also consistent with the supposition of a slow release of a toxic antigen from a depot. Holt (1919, 1951) contends that AlPO_4 adjuvants promote the transport of toxoid antigens to the antibody-synthesizing cells; and that, because excision of the inoculation site in guinea-pigs after 14 days did not diminish the later antibody response, the fibrous reaction round the site, far from forming depots for slow release of antigen, must be sufficiently occlusive to prevent any release at all. Freund (1951) confirms this absence of a prolonged depot effect in rabbits given mixtures of his adjuvant and typhoid bacilli; excision of the injection site half an hour and eight days after injection decreased but did not eliminate the adjuvant effect, and excision after 14 days failed to modify it at all.

Slow release of antigen, or at least a more widespread and prolonged stimulus, might be expected to impose a secondary response on what starts as a predominantly primary stimulus (see Holt 1951). However, the merging of a primary and secondary response alone will not in all cases account for the action of an adjuvant. For example, when plain antigen is given intravenously to animals previously injected with adjuvant and antigen, a conspicuous rise in antibody titre occurs, indicating that the antigen slowly released after the first injection had not elicited all the secondary responsiveness of which the animals were capable (Freund and Bonanto 1942, Ward *et al.* 1950).

Mann and Welker (1949) record a curious adjuvant effect that can be induced in dogs by generally intoxicating the animals with egg white or ricin, injected by another route at the time of giving the antigen.

The Negative Phase.—Just as the injection of a considerable dose of a particular antigen into an animal that possesses a corresponding normal antibody may result in a temporary decrease in the concentration of that antibody in the circulating blood, the injection of a large dose of antigen into an animal that has already developed a certain concentration of antibody in response to earlier injections may be followed by a well-marked negative phase, which is usually followed in its turn by a rise in titre above the previous level. An illustrative example is afforded by the response of an immunized rabbit to two injections of horse serum, given intravenously at an interval of 5 days (Fig 262, p 1275). The importance of avoiding this negative phase in therapeutic immunization was emphasized by Wright and his co-workers (Wright 1909), though others maintain that in these circumstances there may be little diminution in the total immunity.

Stavitsky (1952) observed, in rabbits immunized with bovine globulin and given intravenous antigen, a diminution in the blood of specific antibody, granulocytes and complement, and a decrease in the capacity of the R.E. system to remove bacteria from the blood—results which suggest a temporary decrease in the antibacterial defences.

Schutze (1939) infected mice with a dose of *Salm. typhi-murium* sufficient to kill 50 per cent. of the animals in about 14 days. The injection of four substantial daily doses of a specific vaccine of proved efficacy, beginning on the second day of the disease, did not

though nitrogen and amino acids may be present, is not in itself toxic and cannot be regarded as analogous to toxins, hemolysins and similar substances. Rather the capsule appears to function as a defensive mechanism of the bacterium against the phagocytic activity of the leucocytes. Encapsulated bacteria may be ingested by a white blood cell but, instead of being killed and digested, remain within the phagocyte for a time and then may be extruded in a viable condition. The ability of an encapsulated bacterium to resist phagocytic destruction may, in fact, result in a wider distribution of the microorganism than it might otherwise attain, through transport within the phagocytic cell. It is of some interest in this connection that non-encapsulated, avirulent pneumococci are highly virulent for rabbits deprived of their leucocytes³⁵. It is perhaps suggestive that the polypeptide material making up the capsule of the anthrax bacillus contains d(—) glutamic acid³⁶ (p. 556). Since proteolytic enzymes attack only polypeptides built up from amino acids of the *L*-series, possibly an encapsulated anthrax bacillus would be highly resistant to the digestive enzymes of a phagocytic cell.

Whether the association of virulence and capsule formation may be entirely accounted for on the basis of bacterial defense against phagocytic destruction is not entirely certain, but such defense undoubtedly plays an important part. The presence of antibodies to the capsular substance breaks down this bacterial resistance, and immunization to the capsular material of the pneumococcus, for example, produces just as high a degree of immunity to pneumococcus infection as immunization to the entire bacterium.

Miscellaneous Factors. In addition to these more or less well defined and better known factors, a number of bacteria have been reported to produce substances which may be associated with virulence. Such, for example, are the necrotizing factor, or necrotoxin, produced by some staphylococci, which kills tissue cells, a hypothermic factor produced by Shiga dysentery bacilli, which lowers body temperature, an edema-producing substance formed by pneumococci, substances associated with the endotoxins of some of the enteric bacteria which affect the blood sugar levels of animals, and so on. Unfortunately, the discontinuous character of present information does not support any satisfactory generalization regarding bacterial virulence, but it seems clear that a pathogenic bacterium may have at its disposal a series of mechanisms, in combination peculiar to itself, which make possible a successful invasion of the tissues of the host.

In this connection it should be noted that bacterial virulence may be apparently enhanced by certain substances not of bacterial origin. The presence of silica in the lungs, for example, predisposes materially to pulmonary tuberculosis, the injection of calcium salts with tetanus bacilli aids, in some unknown manner, in the establishing of a nidus of infection. The suspension of bacteria, such as meningococci or typhoid bacilli, in solutions of gastric mucin markedly enhances their virulence, or, more precisely, interferes with the defense mechanisms of the host. It has been found that in the case of the

³⁵ Rich and McKee: *Bull. Johns Hopkins Hosp.*, 1939, 64:434.

³⁶ Bruckner and Ivanovic: *Ztschr. f. physiol. Chem.*, 1937, 247:281, *Ztschr. Immunitäts.*, 1937, 90:304, *ibid.*, 1937, 91:175, *Naturwissenschaften*, 1937, 25:250.

The Response of the Antibody-forming Apparatus to the Administration of Antigens otherwise than by Injection into the Tissues.

In defining the properties of an antigen on p. 223, it was stated that it should stimulate the formation of an antibody when introduced *parenterally*

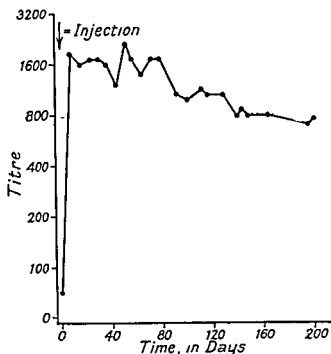


FIG. 260.

Agglutinin production: response of a previously inoculated person to a single injection of typhoid vaccine.

Thus, the administration *per os* of killed suspensions of flagellated bacilli of the typhoid-paratyphoid group results in the production of somatic (O) agglutinins, but flagellar (H) agglutinins are either not produced at all, or only to low titre (Pijper and Dau 1930, Greenwood, Topley and Wilson 1931). The same difference between the formation of H and O agglutinins appears to hold in the case of the cholera vibrio (Pfeiffer and Lubinski 1930). Dysentery bacilli, when administered by the mouth, induced the formation of somatic agglutinins (Kanai 1921, Otten and Kirschner 1927) and of protective antibodies; though with *Sh. sonnei* in mice, a single dose of 10^8 bacilli intraperitoneally proved to be as effective in stimulating their formation as 15 daily doses of 10^9 bacilli by stomach tube (Cooper *et al* 1949). It is probable that analogous effects are produced by many other bacterial cells (see for instance Ross 1926, 1930, 1931, 1932, 1934, Downs and Bond 1937, Moor and Brown 1937, Torikata and Imaizumi 1938). The Vi antigen of the typhoid bacillus appears to be ineffective by the oral route Guarnacci (1939), for example, obtained low H and O titres in 68 per cent. of 227 persons given an oral vaccine of a (Vi + O) strain, but no Vi agglutinins.

In man, Greenberg and Fleming (1950) induced moderate antitoxin responses by oral diphtheria and tetanus toxoids, giving doses of 2,000 Lf or more of each antigen.

In all cases it would seem that administration *per os* is far less effective than inoculation into the tissues, in the sense that much larger doses have to be given and the antibody titre attained is usually lower; and that it has little practical value in medicine (Dolman 1948).

As regards other routes of entry, Stillman (1927, 1930) has shown that protective antibodies are produced in rabbits after the administration of living pneumococci by inhalation, but here we may be dealing with an active infection and consequent tissue invasion by living bacterial cells.

into the body. This caveat was included in the definition because administration *via* the intestinal tract does not ensure the passage of the antigen to the tissues in an unaltered and effective form. It may be broken down by the digestive enzymes. If it is not acted on by them it may never pass through the intestinal mucosa. Whether, in fact, any particular antigenic material administered by this route reaches the tissues in an unaltered form can only be determined by direct experiment. The available evidence suggests that some bacterial antigens, but not all, induce the formation of specific antibodies when administered by the mouth.

meningococcus the mucin interferes with the bactericidal action of the body fluids and, when mixed with peritoneal fluid, provides a medium for its growth.³⁷

Relative Pathogenicity of Bacteria. From the foregoing considerations it will be clear that the virulence of a bacterium is an expression of the efficacy of the aggregate of offensive mechanisms that it may possess. It is to be expected, then, that the bacteria constitute a continuous series of types ranging from those which are unable to infect higher organisms, such as the autotrophic forms, to those which produce a fulminating, widely disseminated and often fatal infection. The harmless saprophyte, *Bacillus subtilis*, for example, has been found to cause, on occasion, an eye infection in human beings (see also p. 565), and the saprophytic microorganism, *Bacterium prodigiosum* which is frequently used as a test organism in part because of its non-pathogenicity, has been found to produce disease following inhalation.³⁸ Somewhat more pathogenic is *Clostridium botulinum*, an organism which is also a saprophyte but which produces a highly potent soluble toxin. The bacterium is itself unable to infect the animal body; the toxin is formed outside the body and gives rise to disease only when ingested in a preformed state. *Clostridium tetani* is one step higher in the scale of pathogenicity; this microorganism resembles the botulinus bacillus in that it is essentially a saprophytic form that produces a soluble toxin. In this case, however, the bacterium is able to establish a nidus of infection in the tissues, albeit in a strictly localized area, and produce the toxin whose absorption gives rise to the symptoms of the disease. The diphtheria bacillus likewise generally produces only a local infection from which its toxin diffuses into the animal body but, unlike the tetanus bacillus, does not lead a saprophytic existence in nature but must maintain a close association with its host. The highly invasive bacteria, such as certain strains of streptococci and staphylococci, are, however, disseminated rapidly throughout the body, presumably because of their armament of fibrinolysins, spreading factor and the like.

A variety of types of infection may, therefore, be distinguished. A bacterium having but small ability to spread through the tissues produces a *localized or focal* infection. Such, for example, are the abscesses at the roots of the teeth, the infection of the heart valves in bacterial endocarditis, etc. A local infection is not necessarily of small consequence; not only may the local tissue destruction be highly significant to the host, as in pulmonary tuberculosis, but, when the microorganism produces a soluble toxin, a poisoning or *toxemia* results, as in diphtheria or tetanus.

Many pathogenic bacteria exhibit a tendency to localize in one tissue in preference to another, sometimes termed *elective localization*; the meningococcus, for example, most frequently localizes in the central nervous system, as do the so-called "neurotropic" viruses, and some of the streptococci show similar preferences for the joints. In some cases the tendency to localize is so marked that a pathogenic bacterium is infectious by one route but not by another, microorganisms such as streptococci, pneumococci, the tetanus bacillus and

³⁷ MacLeod Amer Jour. Hyg., 1941, Sec B 34:51. See also Olitski Bact. Rev., 1948 12:149.

³⁸ Paine Jour Inf Dis., 1946, 79 226.

or other cells. We have discussed in Chapter 7 the validity of the hypothesis that only proteins or protein-containing substances can induce an antibody response when injected into the tissues. It is clear that protein-free derivatives of fully antigenic substances not only act as haptens, but in some cases are themselves fully antigenic. There can be no doubt of the validity of Landsteiner's broad distinction between complete antigens and haptens, or of the association between decreasing chemical complexity, or molecular size, and a progressive loss of antigenic function; but it is not possible to predict at what stage in the degradation of a given antigen the complex will lose its full antigenic powers. In this connection it is instructive to consider the main type-specific antigen of the pneumococcus.

All the early studies on the purified polysaccharide components isolated from various bacteria, and in particular from pneumococci, suggested that these substances had the properties of haptens, and induced no immunizing or antibody-forming response when injected into the tissues. But subsequent observations have disproved this view.

Schiemann and Casper (1927) reported that the purified specific polysaccharide of the Type I pneumococcus, when injected into mice, produced an active immunity against living Type I pneumococci, but not against other types. They noted that this immunity was induced by the injection of small, but not of larger, doses. Schiemann (1929) recorded the immunization of mice by the injection of Type II pneumococcal polysaccharide, and noted that the blood of these immunized mice contained protective antibodies; though he was unable to demonstrate antibody production in rabbits. Francis and Tillett (1930) recorded observations suggesting that persons who were given repeated intradermal injections of the purified polysaccharide of Type I, II or III pneumococcus developed a specific local response which took the form of an immediate wheal and erythema; and that this response was associated with the appearance of specific antibodies in the blood. Similar observations have been reported by Finland and Sutliff (1932) and by Zozaya and Clark (1932). Zozaya and Clark (1933) recorded the immunization of mice with polysaccharide fractions from Types I, II and III pneumococci, and noted that small doses were more effective than larger ones; but their results were irregular except when the polysaccharides were adsorbed on collodion particles or on carbon.

Later studies by Avery and Goebel (1933) have thrown further light on this question. They were able to show that the Type I pneumococcal polysaccharide, as it occurs in the bacterial capsule, possesses acetyl groups. These groups are lost during the separation and purification of the polysaccharide by the methods which have been commonly employed. The acetylated form was found to induce active immunity in mice, when it was administered in small, but not in large doses. It did not induce antibody production in rabbits. Pneumococcal polysaccharides of various types were subsequently shown to be highly effective in inducing the formation of protective antibodies in man (Heidelberger *et al.* 1946, 1950). Analogous results have been recorded in animal experiments with substances from other bacteria. For example, in *Salm. typhi* and *Br. melitensis* the lipopolysaccharide O-substance is fully antigenic; whereas in *Sh. dysenteriae* the antigenicity of the O-substance is lost when the association between the polypeptide and lipopolysaccharide moieties of the molecule is destroyed (Chapter 8).

The Response of the Antibody-forming Apparatus to Active Infection.

The examples given above all refer to the response of the antibody-forming apparatus to the experimental injection of non-living antigenic substances. We may close this section with a few examples of its response to natural infections of the clinically obvious type. For simplicity we may confine our attention to acute infections, noting, however, that in the case of subacute and relapsing diseases,

others are relatively harmless when swallowed but regularly produce infection when injected into the tissues of a susceptible animal. Others, such as the organisms causing typhoid fever, cholera, the dysenteries and other enteric infections, are harmless when rubbed into the abraded skin but promptly produce disease when swallowed by man.

Bacteria present in a primary focal infection may spread by metastasis to set up multiple secondary foci, a condition known as *pyæmia*. Other bacteria may multiply in the blood stream, a condition known as *bacteræmia* or *septicæmia*, the invading bacteria may become widely disseminated through the capillaries of the tissues with the production of a fulminating, *generalized infection*.

Infection is, however, in part a function of the defensive mechanisms of the host, and in some cases a bacterium capable of further spread is held in check by these defenses and sets up a *latent infection* which may flare up when resistance is reduced. Such an inconclusive outcome may result in the *carrier state*, a condition in which a virulent bacterium, fully capable of producing disease, is harbored by a healthy and unaffected host. In this latter instance, however, the resistance of the host is not infrequently specific and a consequence of previous immunization.

Mixed and Secondary Infections. It has long been known that an individual might be attacked by two or more infective agents at one time. Diphtheria and scarlet fever, syphilis and gonorrhœa, pneumococcus pneumonia and typhoid fever, are combinations by no means unknown. It is possible that in some cases the different infections may originate nearly simultaneously, but such an occurrence is probably not common. Usually one infection precedes another, and the second is very frequently a more or less direct outcome of the first. Infection with certain microorganisms predisposes to secondary infection with the pneumococcus; acute tuberculosis may develop during an attack of measles, streptococcus invasion of the lung tissues is not uncommon in pulmonary tuberculosis. The secondary invader is commonly present in the host, but seems incapable of initiating an infection until the host defenses are weakened by the primary disease. Certain microorganisms that can cause primary infection are also frequently found as secondary invaders; pneumococci and streptococci are preeminent in this respect, and show a remarkable capacity for invading the body in the wake of other microorganisms.

Mixed infections of a somewhat different sort are those in which the principal pathogenic organism is accompanied by auxiliary bacteria, or, as some French bacteriologists have called them, *accomplices*, which by their presence influence the virulence of the chief infectious agent without themselves taking any very active part in the infectious process. Such cooperation is, of course, an instance of bacterial synergism which has been discussed previously. The aerobic bacilli which usually enter a wound along with tetanus bacilli probably facilitate the growth of the latter by providing anaerobic conditions. In other cases of mixed infections the invading bacteria may not influence one another directly but only indirectly in a joint breakdown of the defenses of the host; such is presumably the case in mixed infections of diphtheria bacilli and streptococci, and there is reason to believe that such a mixed infection is more severe than an infection with diphtheria bacilli alone—possibly spreading factor

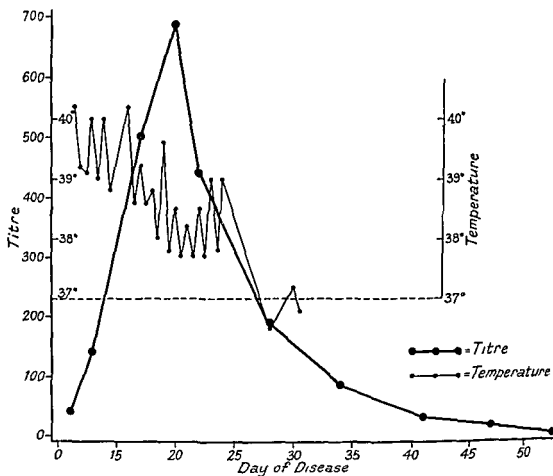


FIG. 263.

Rise and fall of agglutinins in blood of patient during an attack of typhoid fever.
(After Jørgensen.)

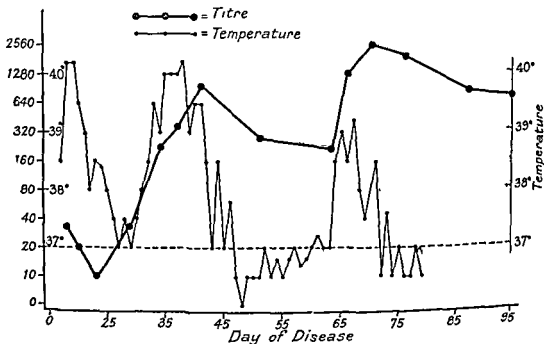


FIG. 264.

Fluctuations of agglutinins in blood of patient during attack of typhoid fever, followed by two relapses.
(After Jørgensen.)

formed by the streptococci increases the permeability of the tissues to the toxin formed by the diphtheria bacilli.

RESISTANCE

It is already apparent from the foregoing considerations of bacterial virulence that, although the ability of a microorganism to produce disease is conditioned by a series of mechanisms originating with the bacterium, pathogenicity must be evaluated in terms of the resistance of the host. As a rule, a pathogenic bacterium is limited to a small number of hosts; bacteria pathogenic for animals are not ordinarily pathogenic for plants; very few of the bacteria that can infect mammals are also pathogenic for cold-blooded animals; some are even restricted to the tissues of a single species. Resistance, like virulence, is made up of many factors, some of which are known either in more or less specific form or in the terms of generalities that serve as a cloak for ignorance; others are, in all probability, as yet unsuspected. Resistance to infection is, in a sense, somewhat more complex than virulence for, as will appear, not only are there specific barriers to infection variable with respect to species and even from one tissue to another in the body of a single animal, but the efficiency of these barriers is also a manifestation of general physiological well-being and hence they are subject to extrinsic or environmental influences.

The differentiation of resistance from natural immunity (p. 327) cannot be made with precision, for the two merge with one another and with acquired immunity without sharp lines of demarcation. In a general way they may be separated on the basis of specificity, resistance being non-specific with respect to the invading microorganism while immunity is sharply specific for a given parasite. The non-specific cellular response (p. 322) is intermediate between the two since the specific cellular immunity of the immunized animal is qualitatively the same, differing only in that it is markedly accentuated.

As in the case of virulence, the better known components of resistance are best considered one by one.

Species, Racial and Inherited Resistance. Species of higher organisms differ greatly from one another in their resistance to any given disease, a fact that has been suggested earlier in connection with the experimental reproduction of disease. In many cases resistance to infection is relative, for disease may sometimes be produced by the administration of massive doses of bacteria to a resistant animal, but in others it appears to be absolute. Man is, for example, apparently completely immune to cattle plague, and many of the lower animals are equally resistant to some diseases of man. In general the factors underlying differences in species resistance are unknown, but in a few cases body temperature or differences in anatomical structure have been found to account for the observed variation. Pasteur's classic experiment in which he rendered the naturally resistant hen susceptible to anthrax by chilling it in cold water, and the converse of this experiment, the production of anthrax in the resistant frog by raising its body temperature to 25° to 35° C., may be explained in terms of unfavorable body temperatures. Similarly, cold-blooded animals are not susceptible to human tuberculosis and warm-blooded animals are not infected by the tubercle bacilli of cold-blooded animals. The insuscep-

Another procedure that has been stated to supply a non-specific stimulus to the antibody-forming apparatus is the injection of various metallic salts, colloidal solutions or other substances. We may ignore, from our present point of view, those instances in which the effect of such injections is limited to an increase in the bactericidal power of the blood. Such effects will be more conveniently considered in Chapter 52.

Walbum and his collaborators (Walbum and Mørch 1923, Walbum and Berthelsen 1925, Walbum 1925, 1926, Schmidt 1926, Ørskov and Schmidt 1928) have recorded numerous experiments along these lines. They conclude that the injection of salts of certain metals, especially those of cobalt, manganese and beryllium, causes an increase in the production of antitoxin, or of agglutinins, if given early in the course of immunization, and leads to a secondary rise in titre if injected when the antibody titre has commenced to fall. Several investigators have, however, failed to confirm these observations (McIntosh and Kingsbury 1924, Horgan 1925), and the protocols included in some of Walbum's reports suggest that the response is very irregular.

Steabben (1925) used agar, gelatin, a silver sol and an iron sol, and found that the injection of these colloids had no power to increase the antibody titre when this had become steady, though when injected at the same time as the bacterial antigen they might increase the antibody response. This last effect is clearly analogous to the adjuvant action of certain substances we have already discussed.

Obermayer and Pick (1904) recorded that animals immunized 3 months previously showed a fresh formation of precipitin after the injection of a 5-10 per cent. solution of peptone. Fleckseder (1916) stated that the injection into human beings of deuterio-albumose or nucleic acid gave rise to a secondary rise in antibody titre. Also Weichardt 1922 and Wahl 1935 have recorded a secondary rise in antibody titre. Both review the evidence on protein substances as non-specific stimuli; in most cases the stimulus is irregular, and the antibody response is small compared with that to a specific antigenic stimulus.

We may next consider those cases in which the non-specific stimulus is provided by a complex antigen, similar in type to that providing the initial specific stimulus but possessing no known common antigenic factor. So far as this antigenic dissimilarity is in fact complete the case does not differ in its essentials from those we have considered above.

Dreyer and Walker (1909) recorded a secondary rise in agglutinins for *Bact. coli* after the intraperitoneal injection of killed staphylococci into previously immunized rabbits. Conradi and Bieling (1916) state that rabbits immunized against *Salm. typhi* show a secondary rise in agglutinins after various non-specific stimuli, such as those provided by the injection of *Bact. coli*, dysentery bacilli and *C. parvulus*. They also injected rabbits with typhoid, paratyphoid and dysentery specific stimulus and another as the secondary non-specific stimulus, and noted many instances of a secondary rise in agglutinins. His protocols, however, indicate clearly that, where the secondary response was well marked, there was some antigenic relationship between the two kinds of bacteria employed.

Rosher (1924) was unable to detect non-specific stimulation of antibody production in tests with the following pairs of dissimilar antigens, the first given as a primary antigenic stimulus, the second as a non-specific stimulus: *Bact. coli* and *Staph. aureus*; *Bact. coli* and sheep red cells; *Salm. paratyphi A* and a suspension of mixed unrelated bacteria; *Salm. typhi* and *Salm. paratyphi B*, *Salm. enteritidis* and living tubercle bacilli. The second injection acted only as a primary stimulus by the antigen used, and did not affect the titre of specific antibodies for the previously injected antigen.

tibility of experimental animals such as guinea pigs and rabbits to the enterotoxin produced by some bacteria is possibly attributable to their lack of a vomiting mechanism. As indicated earlier, resistance to a given infectious agent is not necessarily associated with phylogenetic relationships, and there is no pattern from which the resistance or susceptibility of an animal can be predicted by logical processes; the tabulation of animals susceptible to a given disease represents information acquired largely by trial and error.

Domestic and Experimental Animals. Not only do species of higher organisms differ in their resistance to infectious disease but the races comprising a susceptible species likewise appear to differ among themselves. There are many instances of differences in resistance to infectious disease in varieties or strains of animals. The relative resistance of Algerian sheep to anthrax is well known and inbred Berkshire swine have been found to be highly resistant to brucellosis.³⁹ That this is a true racial immunity which is, as might be expected, inheritable, has been demonstrated by extensive experimental investigations with laboratory animals. The earlier studies of Wright and Lewis⁴⁰ showed that marked differences in susceptibility to tuberculosis existed between inbred families of guinea pigs, differences which were transmitted to the offspring. Later work, summarized by Webster,⁴¹ Hill⁴² and Greenwood *et al.*,⁴³ has been confined, for the most part, to studies on the susceptibility of mice to infection with *Salmonella typhi-murum* and similar bacteria, it has been shown that resistance to such infections may be raised or lowered by selective breeding, sometimes to a remarkable degree. Similarly, strains of mice differ in their susceptibility to murine typhus, and Lurie⁴⁴ has bred strains of rabbits resistant and susceptible to infection with tubercle bacilli. It is of interest that resistance to bacterial endotoxin may also be raised or lowered by selective breeding. Resistance does not behave as a simple Mendelian character but is to some extent specific in that a race having increased resistance to infection with one microorganism is not necessarily unusually resistant to another. For example, in Webster's work mice selected for resistance to infection with *Salmonella enteritidis* showed increased resistance to pneumococcus and Friedlander's bacillus infection, but were more susceptible to the virus of louping ill than the strain selected for susceptibility.

Considerable interest has attached to the mechanisms underlying inherited resistance and susceptibility, and in recent years suggestive results have been reported by a number of workers. Thus, in Lurie's⁴⁴ studies resistance was associated with low skin permeability as assayed by intradermal inoculation of india ink, increased rate and intensity of antibody (agglutinin) response, and the development of a high degree of hypersensitivity (see also p. 343). Gowen and Calhoun⁴⁵ have shown that in mice there is a marked correlation

³⁹ Cameron, Gregory and Hughes: *Amer. Jour. Vet. Res.*, 1943, 4: 387.

⁴⁰ Wright and Lewis: *Amer. Naturalist*, 1921, 55: 20.

⁴¹ Webster: *Medicine*, 1932, 11: 321.

⁴² Hill: *Medical Research Council, Special Report Series*, No. 196, 1934.

⁴³ Greenwood, Hill, Topley and Wilson: *Medical Research Council, Special Report Series* No. 209, 1936.

⁴⁴ Lurie: *Amer. Rev. Tuberc.*, 1941, 44: Suppl.

⁴⁵ Gowen and Calhoun: *Jour. Inf. Dis.*, 1943, 73: 40.

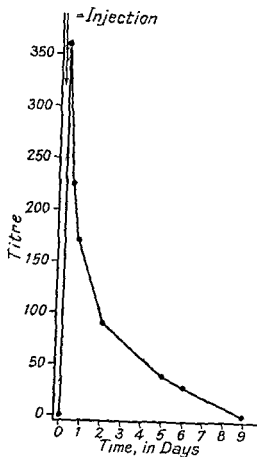


FIG. 265.

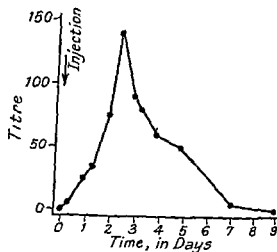


FIG. 266.

Rise and fall of agglutinins in blood of a rabbit after subcutaneous injection of agglutinating serum.

(After Smith.)

(After Smith.)

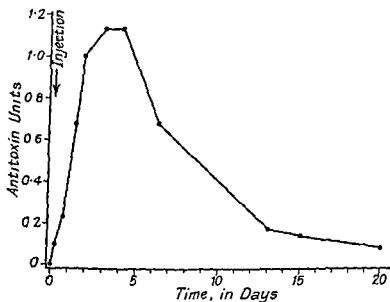


FIG. 267.

Rise and fall of antitoxin in blood of human subject after subcutaneous injection of antitoxic serum.

(After Smith.)

between numbers of leucocytes and resistance to mouse typhoid. Similarly, Severins, Roberts and Card⁴⁶ have found that resistance and susceptibility of breeds of chickens to *Salmonella pullorum* infection is associated with numbers of lymphocytes, and Oakberg⁴⁷ has observed differences in liver and spleen in resistant strains of mice associated with the ability of macrophages to digest phagocytosed bacteria. Differences in susceptibility may, of course, be a reflection of corresponding differences in immunizability, i.e., ability to respond to antigenic stimuli with antibody production. Scheibel,⁴⁸ for instance, has been able to divide strains of guinea pigs into good and poor producers of diphtheria antitoxin on immunization.

Races of Man. The relative resistance of races of man to infection has been the subject of considerable interest and such investigation as has been possible. Under ordinary circumstances in this country the non-white races are much more susceptible to infectious disease than the white race. There are, however, certain exceptions. Thus, the influenza epidemic of 1918 appears to have had a greater impact upon the death rate for white youths than upon that for the non-white population in the same age group. Similar exception may be noted in the case of Baltimore Negroes who showed a lower ratio of clinical diphtheria to immunizing infections than corresponding white children.⁴⁹ It is quite generally recognized, too, that the Negro has a remarkable degree of resistance to erysipelas, and the more favorable response of the Negro to all forms of treatment for gonorrhoea is well known.

Special interest has attached to the white and non-white tuberculosis death rates, both crude and age-specific (p. 643). Whether the observed high mortality in the non-white represents a racial susceptibility or is entirely a reflection of economic status has been the subject of considerable discussion. Studies on this question made in the Army (p. 644) would seem to indicate a true racial difference.

There also appear to be differences between the less well-defined "races" of man. There is evidence that the Irish are less resistant to tuberculosis than certain other elements of the American population, such as the Italians.⁵⁰ On the other hand, the Jewish race is considered by many to be relatively resistant to tuberculosis; in spite of a high incidence of infection, the mortality is very low.⁵¹ To what degree the evidence supports the hypothesis that races⁵² of man differ in their susceptibility to this and other diseases, such as pneumonia, is problematical, for adequate control of the environmental factors is difficult if not impossible. However this may be, twin studies on tuberculosis indicate the operation of genetic factors in the resistance of man to this disease,⁵³ and

⁴⁶ Severins, Roberts and Card: Jour. Inf. Dis., 1944, 75:33.

⁴⁷ Oakberg: Jour. Inf. Dis., 1946, 78:79.

⁴⁸ Scheibel: Acta Path. et Microbiol. Scand., 1943, 20:464.

⁴⁹ Frost: Jour. Prev. Med., 1928, 2:325.

⁵⁰ Guilfooy: Quart. Publ. Amer. Stat. Assn., 1907, 10:515, Dublin: Amer. Econ. Rev., 1916, vol. 6, no. 3, Dublin and Baker: Quart. Publ. Amer. Stat. Assn., 1920.

⁵¹ For example, see Drolet: Amer. Rev. Tuberc., 1924, 10:280.

⁵² In a number of these studies racial stocks were determined for first generation immigrants on the basis of parental birth place.

⁵³ Cf. Kallman and Reisner: Amer. Rev. Tuberc., 1943, 47:549.

from that of an intravenous injection. After an intramuscular injection an anti-serum usually reaches the blood stream more slowly than after an intraperitoneal injection but more rapidly than after a subcutaneous injection; though Christensen (1952) found substantially equal rates of absorption in man and rabbits given tetanus antitoxin by the subcutaneous and intramuscular routes. The rate of absorption from subcutaneous and intramuscular injection sites is increased when the antibody is given together with hyaluronidase (McClellan and Morgan 1933, Boquet *et al.* 1952).

Another point of some importance in connection with passive immunization is illustrated in Figs. 268 and 269 (Glenny and Hopkins 1922).

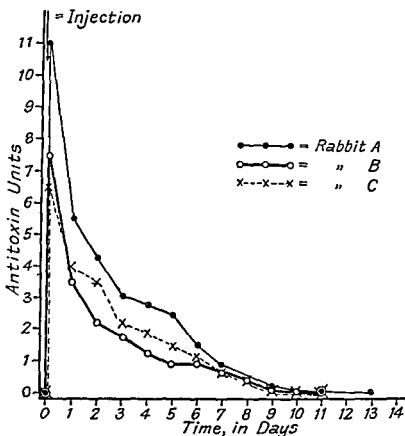


FIG. 268.

Disappearance of antitoxin from blood of three normal rabbits, after intravenous injection of antitoxic horse serum.

(After Glenny and Hopkins.)

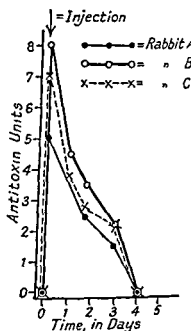


FIG. 269.

Disappearance of antitoxin from blood of three rabbits, previously sensitized to normal horse serum, after intravenous injection of antitoxic horse serum.

(After Glenny and Hopkins.)

In Fig. 268 is recorded the rate of disappearance of antitoxin from the circulation of 3 normal rabbits after the intravenous injection of 0.5 ml. of diphtheria antitoxin, derived from the horse. Fig. 269 gives a similar record in the case of 3 rabbits that had received previous inoculations of horse serum. These were "immune" or "sensitized" to horse serum—the relation of the immune to the sensitized state is discussed in Chapter 51—and in consequence eliminated the antibody from their circulation much more rapidly than did the normal rabbits. The same phenomenon will occur when a person who has previously received an injection of horse serum is given an antitoxic or antibacterial serum derived from that animal (see Hooker and Follensby 1931).

the question is rather one of the occurrence of practically significant genetic segregation.

A given disease, however, may be relatively mild in its effect on races of man which have been in contact with it over a long period of time, but assume a highly virulent form in other races to which it is new. Measles, for example, a mild disease to civilized man, has been a scourge to certain primitive races. In other cases, diseases originally highly virulent have become apparently less so with the passage of time, leprosy is not as widespread as it was in biblical times, and syphilis is a considerably milder disease today than it was in the sixteenth century. Phenomena such as these have been taken by some to indicate the development of a racial immunity through a selection of more resistant individuals, and by others to suggest an adaptation of the microorganism accompanied by a loss of virulence. At the present time, it is not possible to differentiate sharply between these two, possibly both effects are operative.

It should be pointed out in this connection that what might be called a pseudo-racial immunity may be manifested by a race in close association with a given infective agent. Many individuals have the disease, the survivors are immune, and this immunity is passively transferred to the offspring (see p. 333), who are infected before this passive immunity entirely disappears and consequently have the disease in a mild form but become solidly immune. The immunity is passively transferred to the third generation, and the process continues *ad infinitum* as long as the race is in contact with the disease. Some such racial immunity of the adults arises as a result

Age. The effect of the age of an animal on its resistance to infections disease is variable, being clearly apparent in some instances and indistinguishable in others. In many cases there is a direct relation between age and resistance, the young being more susceptible than the older individuals. The suitability of the developing chick embryo as a menstruum for the cultivation of certain filterable viruses to which the chicken is apparently completely insusceptible, and the results of the experiments of Woolpert and his colleagues⁵⁴ on the infection of the guinea pig fetus, may be regarded as examples of an exaggerated susceptibility of the immature organism. The marked susceptibility of the guinea pig fetus to tuberculosis drops off after birth, and young, mature and old animals have been found to be progressively more resistant to this infection. Similar results have been observed with other infections such as equine encephalomyelitis in mice, St. Louis encephalitis in mice, and in the resistance of the rat to diphtheria bacilli and diphtheria toxin.

It is well known that the very young child does not respond well to active immunization, Sauer,⁵⁵ for example, has reported poor results in active immunization against whooping cough, and antidiphtheria immunization of the newborn is not successful. This inadequate immune response is undoubt-

⁵⁴ Cf. Woolpert: Amer. Jour. Path., 1936, 12:141, Woolpert et al.: Jour. Exp. Med., 1938, 68:313, Gallagher and Woolpert: Jour. Exp. Med., 1940, 72:99, Dettweiler, Hudson and Woolpert: Jour. Exp. Med., 1940, 72:623.

⁵⁵ Sauer: Amer. Jour. Path., 1941, 17:719.

structure of antigens and antibodies, was put forward independently by Breinl and Haurowitz (1930), Alexander (1931) and Mudd (1932). Ignoring minor differences in the views of its sponsors, this hypothesis assumes that all antibodies are globulins newly synthesized under the directing influence of the antigen. This influence is exerted through a union, physical or chemical, between the peptides of the nascent globulins and the active groupings of the antigen, during the globulin synthesis. The globulin thus formed is dissociated from the antigen after a certain stage has been reached, and the latter is thus able to leave its stereochemical imprint on successive molecules of antibody globulin synthesized under the same conditions.

We have seen that certain cells of the lymphoid-macrophage system appear to be the site of antibody formation, but the circumstantial evidence does not implicate the cells of that system in which antigen is first deposited (p. 1254). If, however, we can take such cells as a model of the antibody-forming cells—and it is not impossible that some of them are transformed into antibody-forming cells—the localization of antigen—or at any rate of radioactive markers attached to antigens—first in the microsomes and then in the mitochondria of the macrophages of the liver and spleen (Crampton and Haurowitz 1952, Fields and Libby 1952) suggests that synthesis takes place in granules of the cell cytoplasm.

The reaction of antigen, or of an effective derivative of antigen, with the synthetic sites, appears to take place fairly rapidly. The results of transplanting splenic tissue, at varying intervals after exposure to antigen, into a normal animal indicate that the antigen loses its capacity to stimulate cells in the recipient animal within a day or so (p. 1259); and experiments on the time-relations of radiation-sensitivity of antibody formation (p. 1256) suggest that the modification of the synthetic site by antigen is initiated within a few hours. In this connection we may also note that the antigenicity of bacillary bodies is partly destroyed soon after their ingestion by macrophages (Walsh and Smith 1951). But although the antigen, as a substance that will stimulate a response in a second animal, may disappear before antibody synthesis has ceased, it does not follow that it disappears as a determinant of antibody formation (Topley 1930). Moreover, it should be noted that transposed antibody-forming tissue loses its capacity to produce antibody in a few days or weeks. There is other evidence, in short-term tests at least, that antibody formation lasts as long as antigen is present in the body. Thus there is a direct relation between decline of antibody formation and disappearance of antigen in rabbits making a primary response to tobacco mosaic virus (Libby and Madison 1947, Erickson *et al.* 1953).

Pauling (1940) who reviewed the physico-chemical basis of the hypothesis, considers that the difference between normal and antibody globulin lies not in the ordering of the amino-acid residues of the polypeptide chains, but in the manner of the folding of their otherwise similar constituent polypeptide chains, to form polypeptide laminae and ultimately the globular protein. According to Pauling, the ends of the folds, which when packed together will constitute one of the surfaces of the completed molecule, are capable of taking up many configurations, whose variety and stability are determined by the presence of an excess of proline and hydroxyproline residues at the point of folding. When an antigenic substance with a well-defined configuration of active groups is present in the cell, it influences the configuration at the ends of the folded polypeptide chains, thereby inducing the formation of an antibody receptor. Pauling suggested that, if globulin mole-

edly at least partially responsible for infection of the very young with bacteria ordinarily regarded as harmless saprophytes. Cass,⁵⁶ for example, has reported epidemic infection of the newborn (in a maternity ward) with *Bacterium aerogenes* which took a septicemic form, and the *Bacterium coli* infection of foals known as scours is well known.⁵⁷ Complement deficiency in the newborn and less active phagocytosis by Kupffer cells are possibly associated with this failure to respond to antigenic stimuli and bacterial invasion.

The rise in resistance coincident with the development of an animal to maturity has been interpreted by some workers as indicative of a "maturation immunity" in which the ability to withstand infection is associated with the

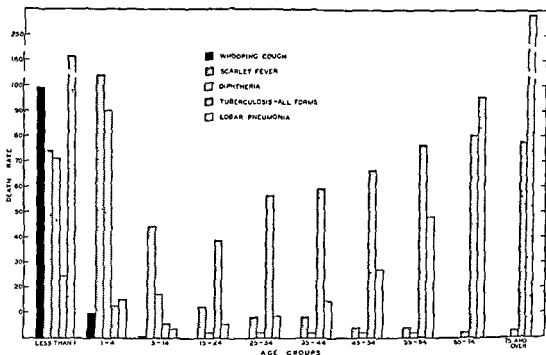


Fig. 29. The age incidence of certain infectious diseases. Note the predominance of the diseases of childhood in the early years, the increase in tuberculosis in the young adult years and the marked increase in lobar pneumonia with advancing age. Scarlet fever rate multiplied by a factor of 40, and diphtheria rate by a factor of 10 for comparative purposes. Data for 1940 from the reports of the Bureau of the Census.

ductless gland secretions. Experimental studies in which precocity has been induced by the injection of androgens have, however, been disappointing, and maturation immunity must be regarded as a hypothesis which is suggested by certain clinical results but for which there is no sound experimental proof.

The well-known increased susceptibility of the aged to certain of the infectious diseases is reflected not only in the incidence but also in the mortality of infections such as pneumonia. The quantitative (exponential) relation between the probability of death from a given disease and age was observed many years ago (1825) and is known as Gompertz's law. The physiological alterations underlying senile debility and mortality are probably in the nature of an accumulation of degenerative changes.⁵⁸

⁵⁶ Cass: *Lancet*, 1941, i:346.

⁵⁷ For instance, see a discussion by Wolfe and Dicks: *Jour. Immunol.*, 1948, 58:245.

⁵⁸ Simms. *Science*, 1940, 91:7.

antigenic units must pass to one of the daughter cells at each division. Burnet and Fenner prefer the assumption of an antibody-forming capacity, independent of the continued presence of antigen, which is transmitted to descendant cells by some hereditary process. (It has been objected that such a process should lead to the hereditary transmission of the capacity to the offspring; but there is no necessary connection between the two, for it is highly unlikely that the immune response in the adult would have any genetic effect on the segregated reproductive cells in the gonads.)

They postulate that the serum globulins are products of self-reproducing, intracellular, globulin proteinases, and represent a stage in synthesis when the molecule has the essential structure of the enzyme, but is without enzymic activity. At this stage it may be turned out into the circulation as a globulin. In the cell, however, the synthesis proceeds to completion, and more proteinase is formed, its normal function being to hydrolyse substances of endogenous origin. In the presence of antigen, the configuration of the proteinase is modified so as to hydrolyse the antigenic matter, and, when the antigen is gone, the proteinase reproduces itself in the modified form, both in the original cell and its descendants. According to Burnet, the continuously accelerated output of antibody observed during the secondary response is characteristic of an autocatalytic process such as he postulates, not the production of antibody from a fixed number of antigenic templates; and he attributes the change in reactivity of antibody observed with prolonged immunization (see Chapter 7) to the profounder modification of the globulin proteinases by the products of a more complete intracellular hydrolysis of the antigen.

That the secondary response is sometimes exponential in part of its course does not necessarily point to any one kind of process, biological or other—a fire in a wood-pile grows exponentially. The exponential increase may, for example, reflect an increase in the antibody-forming cells, thus giving an opportunity to unemployed templates in regions where antigen has accumulated; for, as Haurowitz (1952) points out, even a small dose of intravenous antigen is equivalent to several thousand molecules per liver cell.

The specific immunological paralysis, induced in adult mice by massive doses of pneumococcal polysaccharide (p 1262), and the analogous phenomenon of specific acquired tolerance induced in animals treated with the antigen during their foetal life, are not readily explicable in terms of either hypothesis. But both appear to be associated with persistence of antigen. In the first, persisting polysaccharide was demonstrable, and in the second, the antigens were foreign tissue cells that are either known, or may reasonably be presumed, to persist in the animal receiving them.

We are not in a position to decide between these hypotheses. Both depend upon arguments by analogy with other biological processes, and it is not in all cases clear how relevant these analogies are. It is clearly impossible to prove in all cases that antigen is persisting as such. Heidelberger (1952) indeed suggests that both mechanisms may be operative; and that antigens, like the bacterial polysaccharides, which are not readily metabolized in the body act mainly as continued stimuli, whereas the readily hydrolysed protein antigens, like diphtheria toxoid, induce a heritable change in the synthetic powers of the antibody-forming cell.

Sevag (1951, 1954) developed another concept, based largely upon a study of the immunology of enzymes and their corresponding antibodies, of antigens as specific catalysts of antibody formation. This, it may be noted, is in direct contrast to the views of Burnet and Fenner, who regard antigens as modifiers of the intracellular proteinases.

In practice, the relation of age to resistance to infection is complicated by many factors. The human baby, for example, is relatively resistant to a number of infections for the first six months of life as a result of passive transfer of antibodies from the mother either via the placental circulation *in utero* or by means of colostrum after birth. Similarly, the increased resistance to a disease such as diphtheria which appears with physical development arises primarily as a result of inapparent infection rather than maturity alone. (The so-called natural antibodies are discussed on p. 329.)

Sex. A number of diseases exhibit a difference in sex incidence, pneumonia and epidemic meningitis, for example, are more common in males than females, while scarlet fever, typhoid fever and others occur somewhat more frequently in females. Except for a few years in the forty-one-year period 1900-1940, the female death rate for white youths has been consistently lower than that for males, though the reverse has been true for the non-white population in this country. In the decade 1931-1940 the gap between the female and male in the non-white population was closed and in 1940 the female and male death rates for non-white youths were exactly the same at 5.0 per 1000. Corresponding rates for the white population were 1.4 for females and 2.0 for males. In the case of tuberculosis, variability in the age-specific mortality rates indicates a differential response on the part of the sexes (p. 644). A sex differential is also indicated by the aftermath of the 1918 influenza epidemic, which appears to have had a more unfavorable effect upon female mortality than that of males in the 15 to 24 age group, as indicated by the relatively high female death rate for all racial groups for several years after the epidemic.

It has been suggested that this difference between the sexes might result from resistance having some of the attributes of a sex-linked character, or, since differences in sex incidence are most pronounced during and after puberty, something in the nature of maturation immunity may be involved. There is no sound evidence to support the first of these hypotheses. Regarding the second a certain amount of experimental evidence has been reported, the most precise relating to skin permeability, which has been found to be greater in female rabbits than in males.⁵⁹ It has also been shown by Bennison and Coatney⁶⁰ that female chicks are significantly more susceptible to infection with the chicken malaria parasite, *Plasmodium gallinaceum*, than are males, treatment with male and female sex hormones did not affect the differences between the two. Any explanation of the observed sex differences must, of course, take into consideration other factors such as occupation, risk of exposure and the like.

Climate and Season. That both climate and the season of the year exert marked effects on the incidence and mortality of a number of infectious diseases is well known. In tropical climates, for example, the acute upper respiratory infections are not so common as in the temperate climates, but the dysenteries are more common in the tropics than in the temperate zones. The seasonal incidence of the infectious diseases is also common knowledge.

⁵⁹ Lurie and Zappasodi. *Proc. Soc. Exp. Biol. Med.*, 1939, 42:741, *ibid.*, *Arch. Path.*, 1942, 34:151.

⁶⁰ Bennison and Coatney. *Science*, 1948, 107:147.

the decline in titre of circulating antibodies after a maximum has been reached is usually delayed. An immunized differs from a normal animal, not only in the possession of a particular antibody, but in its capacity to produce that antibody rapidly and in large amount in response to the specific stimulus. In certain circumstances the preoccupation of the antibody-synthesizing cells with the secondary response may diminish their response to a primary inoculation of another antigen made at the same time.

(6) Repeated inoculations are more effective in raising the antibody titre to a high level than a single injection, even of a very large dose. Just as there appears to be a limit to the titre that can be attained by increasing the size of a single dose of antigenic material, so there is a limit beyond which repeated injections fail to raise the titre.

(7) The injection of a large dose of antigen into an animal in whose blood the corresponding antibody is present frequently results in a temporary fall in the concentration of that antibody, followed in most cases by a secondary rise. The efficacy of the dose of antigen in stimulating this secondary rise may be diminished by the combination of antigen with the circulating antibody—a “blanketing” effect apparently due to a consequent accelerated breakdown of antigen in the tissues.

(8) Antibody formation may be induced by the administration of antigens by the mouth, by inhalation, or by intranasal instillation. Administration by these routes is, in general, less effective than direct inoculation into the tissues. In the case of administration *per os* there is evidence that antibodies may be produced against certain antigenic components but not against others.

(9) Although the majority of naturally occurring antigens are proteins, or contain protein components, other substances, such as complex polysaccharides, are capable of inducing an active immunity, and of stimulating antibody production.

(10) The antibody-producing apparatus may in certain conditions be stimulated by non-specific substances. Two effects may be distinguished:

(a) An adjuvant action of substances injected into the tissues simultaneously with antigen, or during the period of increasing antibody response. The action of adjuvants is complex. In some cases, they appear to act by inducing tissue lesions from which the release of antigen is slowed and prolonged; in others, by promoting reactions that lead to a more efficient transport of antigen to the antibody-forming cells. A stimulus to the multiplication of antibody-forming cells cannot be excluded from the effect of certain adjuvants.

(b) A stimulation of the antibody-forming apparatus that results in some increase in the concentration of a normal antibody or in the renewed production or mobilization of an antibody previously produced in response to a specific stimulus—the anamnestic reaction. The evidence for such stimulation is conflicting; this non-specific response often occurs very irregularly, is usually trivial or transitory in its effect, and is never as great as the response to a further injection of the specific antigen. In some cases it may be mediated by hormones of the adrenal cortex.

(11) The rate at which injected antibody reaches the blood and tissue fluids of a normal animal and the concentration there attained depend in great part on the route by which the antiserum is injected. After an initial period of dilution in the blood and body fluids, passive circulation at a rate similar to that

meningococcus meningitis occurs predominantly in the winter and spring, poliomyelitis in the late summer and early fall, and so on. One of the most striking relationships of season to incidence of disease is that of Asiatic cholera; the epidemic season coincides with hot weather and shows a remarkable correlation with precipitation and relative humidity.

In a great many instances the influence of climate and season upon the incidence of disease may be attributed to opportunities for transmission of the causative organism, such as crowding in poorly ventilated rooms, seasonal or geographic occurrence of an insect vector and so on, but in others the factors responsible for seasonal incidence are unknown.

In recent years specific evidence has begun to accumulate which substantiates the opinion that resistance varies with season, temperature and similar factors. For example, the intensity of the brain reaction of mice inoculated with *St. Louis encephalitis virus* and that of guinea pigs inoculated with endemic typhus virus have been found to have been the greatest in summer and the least in the winter over a period of years; mice adapted to existence in a moist heat have been found only one quarter as resistant to infection with hemolytic streptococci as those adapted to a cool environment; the morbidity and mortality rates in murine typhus infection in mice are affected by environmental temperature; and the resistance of mice to pneumococcus infection is similarly affected. It is not improbable that future investigation will yield systematic knowledge of the effect of climatic factors on susceptibility to disease.⁶¹

General Physiological Well-Being. Whatever resistance to disease an organism may possess by virtue of species, race and the like is profoundly influenced by its general physiological state. In general, resistance is at its height when the organism is functioning normally in every respect, and is reduced by a variety of factors which interfere with and alter the normal physiological state. In some cases previous infection may reduce resistance to such a point that infection with the less virulent bacteria may take place, as in the secondary infections; in others, functional disorders such as diabetes mellitus bring about a reduction in resistance to infection. More common, however, are the deleterious effects of inadequate diet and fatigue.

Nutrition. The relation between susceptibility to infection and faulty nutrition has been of considerable interest since the discovery of the vitamins and consequent study of the deficiency diseases, in some of which, such as xerophthalmia, infection plays a part. Attention has been directed particularly toward vitamins A (carotene) and C (ascorbic acid), and there is no doubt that a deficiency of the first of these substances results in a marked lowering of resistance. The reduced resistance appears to be associated with a reduced humoral immune response, but this is in part at least compensated for by an accentuated phagocytic response to infection. Diets qualitatively and quantitatively inadequate, not only with respect to other vitamins but in other ways also, may likewise predispose to bacterial infection.⁶² Recently Cannon⁶³ has

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⁶³ Cannon. Jour. Bact., 1946, 51: 582.

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emphasized the importance of adequate protein intake, and he and his co-workers have shown that depletion of the protein reserves of experimental animals by maintenance on low protein diets interferes markedly with antibody formation, *i.e.*, the synthesis of immune globulin. While antibody formation is significantly interfered with by moderate protein depletion, a severe depletion also interferes with the normal functioning of the cellular defense mechanisms as evidenced by reduced phagocytosis.⁶⁴

Other than this, just how malnutrition brings about a reduction in resistance is not known, present information indicates that the effect is a general one. It is of some interest that this unfavorable effect is not limited to the undernourished individual but may be transmitted to offspring. Experimental studies have shown,⁶⁵ for example, that the diet of mice, genetically homogeneous in resistance to an infection, affects the resistance of offspring to a greater degree than the diet of the offspring themselves. In this connection, statistical studies⁶⁶ have suggested that the mortality rates of adult human life are in part a function of nutritive and other elements of the environment of childhood, in other words, the effect may be a delayed one, but the evidence on this point is not altogether indubitable.⁶⁷

The marked reductions in resistance associated with inadequate diets are in no sense specific; resistance to infection in general is reduced, and there is no relation between lack of a single dietary factor and susceptibility to a particular infection. A number of attempts have been made to demonstrate such a relation, ascorbic acid, for instance, has some small capacity to neutralize diphtheria toxin, but vitamin C deficiency does not predispose to infection with the diphtheria bacillus any more than with other organisms, such as the tubercle bacillus.

Since inadequate diet, and vitamin A deficiency in particular, reduces resistance to infection, it has been supposed that resistance might be raised in hypervitaminosis. Experimental evidence, however, does not support this supposition, the normal level of resistance maintained by the organism on an adequate diet cannot be appreciably raised by the administration of vitamin A in quantities in excess of that normally required.

The apparently contradictory observation that resistance to infection with a number of the viruses is increased by inadequate diet has been made by a number of workers.⁶⁸ That it is not completely inconsistent with the lowered resistance associated with protein depletion and other dietary deficiencies is indicated by the consideration that the viruses are obligate intracellular parasites; possibly a healthy cell is of primary importance to multiplication of the virus and the depleted cell is an unfavorable medium for development, thus masking so to speak the lessened immune response associated with nutritional deficiencies.

Fatigue. It has long been known to the clinician that bodily rest is a valuable adjunct to the treatment of disease, and there is clinical evidence

⁶⁴ Cf. Mills and Cottingham: *Jour. Immunol.*, 1943, 47:503, Guggenheim and Buechler *ibid.*, 1948, 58:133.

⁶⁵ Cf. Church: *Amer. Jour. Pub. Health*, 1939, 29:215.

⁶⁶ Cf. Greenwood: *Jour. Roy. Stat. Soc.*, 1936, 99:674.

⁶⁷ Cheeseman: *Human Biol.*, 1939, 10:537.

⁶⁸ Cf. the summary of the literature by Kearney, *et al.*: *Jour. Bact.*, 1948, 55:89.

which suggests that resistance to the initial infection may be reduced by excessive fatigue. Experimental evidence on this point is scanty and to some degree conflicting, but it is probable that the unfavorable effect of fatigue on normal physiological well-being is reflected to some extent in an increase in susceptibility to infection. The normal white rat, for example, is highly resistant to anthrax, but when exhausted by work in a treadmill becomes susceptible,⁶⁹ latent *Salmonella enteritidis* infections in the same experimental animal may be activated by fatigue to such a degree that the outcome is fatal.⁷⁰ Similarly, studies on human beings have indicated that an individual may be rendered transiently susceptible to the common cold by fatigue.⁷¹

Other mechanisms operative in the resistance associated with general physiological well-being are obscure. Studies on resistance to the common cold in population groups made by subjecting the incidence data to an analysis of variance among groups have strongly suggested that an as yet undefined constitutional factor is operative in the etiology of the clinical infection.⁷² The studies of Locke⁷³ have indicated that the capacity to maintain effective circulation and the ability to withstand the effects of sudden temperature changes are associated with resistance to experimental infection. The adverse effects of sudden changes in temperature and humidity on the organism, reflected in changes in the nasal mucosa, are, perhaps, a manifestation of temperature shock. Attempts to associate such shock, vitamin deficiencies, fatigue and other elements of the non-specific resistance of health with specific defense mechanisms such as the capacity to form antibodies, have not been uniformly successful.

The External Defenses of the Organism. The cellular organization of the animal body is a closed system with respect to the outside environment from which it is separated by the skin, mucous membranes and intestinal mucosa. These structures, generally impermeable to particulate material of the size of bacteria, constitute the first line of defense against invading microorganisms and one that is, for the most part, highly effective. While mechanical obstruction contributes in no small part to the efficacy of these barriers, both skin and mucous membranes also play an active part in the protection of the organism against bacterial invasion, as will appear.

The Skin. As a rule, the unbroken skin presents a more or less impassable barrier to microorganisms. Bacteria are found normally on the skin between the superficial horny cells, but ordinarily are not able to penetrate deep into the tissues unless favored by some cutaneous injury, such as a wound or burn. The ducts of the sweat glands and the hair follicles are, however, vulnerable points, and experiment has shown that it is possible for bacteria to penetrate the skin through these channels.

The skin, however, is not an inert surface upon which bacteria may survive, but is, as experimental work of the last few years has shown, actively bactericidal. Arnold and his co-workers⁷⁴ found that bacteria which do not

⁶⁹ Charrin and Roger: Arch. Physiol. Norm. Path., 1890, 22:273.

⁷⁰ Boycott and Price-Jones: Jour. Path. and Bact., 1926, 29:87.

⁷¹ Locke Jour. Immunol., 1939, 36:365.

⁷² Jour. Hyg., 1947, 45 29.

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occur normally on the skin, such as *Bacterium prodigiosum*, the typhoid, colon and enteritidis bacilli, and hemolytic streptococci, are rapidly (in some cases within ten minutes) destroyed when swabbed on the clean skin of the palm of the hand. Possibly related is the fungistatic action of the free saturated aliphatic acids in the hair fat of adults.⁷⁵ The autosterilizing capacity of the skin is markedly reduced by dirt and is apparently a property of live skin, the skin of a cadaver dead only fifteen minutes showed little or no bactericidal activity. Bacteria normally present upon the skin, such as the white staphylococci, are not appreciably reduced in numbers when swabbed on the clean skin, a fact which probably accounts for their constant presence on the body.

The Conjunctivae. Bacteria and dust particles settling in the eyes are removed relatively rapidly by the mechanical flushing effect of the tears. The lacrimal secretions contain a substance, *lysozyme*, which is also present in certain tissue extracts and in egg white, which destroys some species of saprophytic bacteria. Fleming⁷⁶ who discovered lysozyme, described one species of bacteria, *Micrococcus lysodeikticus*, which is particularly susceptible to the action of this lytic substance, as high a dilution as 1:40,000 of tears has brought about complete lysis of these microorganisms. Lysozyme has been found to be identical with the avidin of egg white and has been isolated in crystalline form.⁷⁷ None of the pathogenic forms, however, appears to be unusually susceptible to the action of lysozyme, and it probably plays no important part in the protection of the organism against invasion.

The Nose, Nasopharynx and Respiratory Tract. Bacteria and other particulate material present in inspired air are rapidly removed by passage through the tortuous nasal passages lined with mucous membrane to whose moist surface they cling. In this way air is largely freed from bacteria in the upper respiratory passages, those that pass the larynx are caught in the bronchi and few reach the ultimate ramifications of the bronchioles. The process is so efficient that expired air contains almost no bacteria except those that are expelled in droplets by sneezing, coughing and talking.

The moist film which covers the mucosa of the upper respiratory tract and in which bacteria removed from inspired air and those arriving via the lacrimal secretions are embedded consists of mucus, a thin, highly viscid substance that, in a sense, constitutes a continuous web or membrane overlying the surfaces within the nose, sinuses, pharynx and esophagus. This film of mucus is in constant motion as a result of the activity of cilia which sweep the mucus and its bacterial content toward the oropharynx, where it is swallowed. The exchange of mucus is rapid, that covering the posterior two thirds of the nose is replaced every ten or fifteen minutes, while that over the anterior third is removed every hour or two.⁷⁸ Although mucus itself has no bactericidal activity, when combined with ciliary activity it constitutes a remarkably efficient means of ridding the upper respiratory passages of bacteria. Davies,⁷⁹ for instance, has shown that all particles above 7 μ in diameter

⁷⁵ Rothman, Smiljanic and Shapiro: *Proc. Soc. Exp. Biol. Med.*, 1945, 60:394.

⁷⁶ Fleming: *Proc. Roy. Soc.*, 1922, Series B, 93:306, *Lancet*, 1929, 1:217, *Proc. Roy. Soc. Med.*, 1932, 24 (See Path.) 1. See review by Thompson: *Arch. Path.*, 1940, 30:1096.

⁷⁷ Alderton, Ward and Fevold: *Jour. Biol. Chem.*, 1945, 157:43.

⁷⁸ Cf. Hilding: *Ann. Int. Med.*, 1932, 6:227.

⁷⁹ Davies: *Proc. Roy. Soc. Ser. B*, 1946, 133:282.

an interval of 10 days elapsed after the sensitizing dose before hypersensitiveness was established; and that no hypersensitiveness resulted when large injections of serum were given at short intervals.

The same phenomenon was studied independently by Rosenau and Anderson (1906, 1907), who established the strict specificity of the reaction. They found that guinea-pigs sensitized to horse serum showed little if any hypersensitiveness to the serum of other animals, such as the rabbit, cat, dog, pig, sheep, chicken or man. Similarly, guinea-pigs sensitized to horse serum did not respond in any abnormal way to the subsequent injection of egg white, vegetable protein or milk. They noted also that the amount of serum required to produce sensitization was extremely small; as little as 0.000,001 ml. sometimes induced typical hypersensitiveness. The amount required to produce anaphylactic shock in a sensitized animal was, however, considerably greater (0.01-0.1 ml.). They confirmed the existence of a latent period of the anaphylactic reaction, extending over an indefinite period, extending over several months at least.

These observations have been repeatedly and consistently confirmed by later workers. Animals may be actively sensitized by the injection of any substance that is antigenic in the full sense. The sensitizing injection may be given by any route. Often a single sensitizing dose suffices. In some animals, and with some antigens, it is necessary to give more than one sensitizing injection, and to use doses far larger than the minute amount that suffices in the particular case of the sensitization of the guinea-pig to horse serum; but as a rule the injections must not be too large, nor too often repeated. Large doses, however, must be used for animals, like the mouse, that are refractory to sensitization. The dose needed may also vary with age; for the one-year-old guinea-pig it is reported to be twenty times that for animals up to 22 weeks old (Coulson and Stevens 1949).

Sensitization is also possible by the oral route (Winter 1944) or by the inhalation of antigen in the form of an aerosol (Kallos and Pagel 1937).

As regards the shock-inducing, or "shocking" injection, either the antigen used for sensitization, or certain types of separated haptens containing the specific reactive grouping, are effective in eliciting the characteristic symptoms. The route of injection is important. Intravenous inoculation gives the most constant results; in some animals it is the only effective route for this purpose. In the guinea-pig, which is peculiarly susceptible in this respect, shock may be produced by intraperitoneal inoculation, but larger doses are required. Within certain limits the size of the shocking dose, nevertheless, appears to be a secondary factor in determining the severity of shock. In dogs, for example, Dragstedt (1943b) found that shock did not become more fatal with increase in size of the shocking dose; the fatality was primarily determined by the degree to which the animal had reacted to the sensitizing dose. The generalization, based on older observations, that the shocking dose must be considerably larger than the minimal sensitizing dose, is not justified. A shocking dose of 0.5 μ g ovalbumin is reported as sufficient in guinea-pigs sensitized with 1 μ g. of the antigen (Coulson *et al.* 1949).

A curious and significant fact emerged early in the widespread investigation which followed the opening-up of this new field of research. The symptoms and lesions of anaphylactic shock were found to be constant for a given animal species, irrespective of the nature of the sensitizing antigen; whereas these symptoms and lesions differed sharply from one animal species to another. Thus the guinea-pig reacts in the same way when anaphylactic shock is produced with horse serum,

which are inhaled are retained in the upper respiratory passages of the rabbit, about half the particles $3\ \mu$ in diameter are similarly removed, and the remainder plus practically all those $1.5\ \mu$ in diameter and smaller penetrate into the lungs. Substantially the same results have been reported by Boyland, Gaddum and McDonald⁸⁰ with other experimental animals and man. The bacteria that penetrate the upper respiratory passages and lodge in the bronchi and bronchioles are probably phagocytosed by the fixed aveolar epithelial cells and the wandering leucocytes that enter the bronchioles and sacs, a process discussed at greater length elsewhere.

Lysozyme is, of course, present in the nasal mucus and it has been observed⁸¹ that the normal serous secretion of the nose contains a virus inactivating agent (VIA) distinct from lysozyme. The activity is virucidal for the influenza and certain other viruses inactivated by sodium desoxycholate but what part it plays in resistance to infection is not clear.

The Mouth, Stomach and Intestinal Tract. The healthy mouth ordinarily contains great numbers of bacteria, but, except in the case of those organisms which have established themselves, this flora is a transient one, the microorganisms being constantly removed through a flushing action of saliva and as constantly supplemented by fresh contamination. Saliva is but mildly bactericidal,⁸² and the removal of bacteria is practically entirely a mechanical process. The microorganisms flushed to the back of the mouth meet with those from the nose and, with them, are swallowed.

Bacteria reaching the stomach are subject to the strongly acid environment of the normal gastric juice, and there is no doubt that the great majority of them are destroyed there. Some do, however, reach the intestinal tract, perhaps because they are embedded in solid particles of food and thus protected or because they are able to withstand a short exposure to the bactericidal action of the gastric secretions. Generally, very few viable bacteria are found in the stomach, but the numbers of microorganisms increase in the small intestine with the rise in pH from the duodenum to the ileum. The large intestine contains great numbers of bacteria derived not only from the upper levels of the intestinal tract but also from the multiplication of bacteria present in the intestines as normal inhabitants. As in the respiratory passages, mucus plays an important part in the mechanical removal of bacteria. Here, however, the mucus does not form a uniform coating over the intestinal mucosa but is present as a meshwork. The villi free themselves from particles by movements which bring them in contact with the mucus to which the particles, including bacteria, adhere. The mucus, with the embedded microorganisms, is rolled up into small masses and moved outward by the peristaltic movements of the bowel.⁸³ Bacteria, then, which enter the mouth and upper respiratory tract are eventually extruded with the feces.

The Genital Tract. The normal genital tract is remarkably free from bacteria. The urethra in both male and female is normally sterile, a consequence,

⁸⁰ Boyland, Gaddum and McDonald Jour Hyg., 1947, 45 290

⁸¹ Burnett, Lush and Jackson Brit. Jour. Exp. Path., 1939, 20 377; Francis Science, 1940, 91 198

⁸² Cf. Van Kesteren, Bibby and Berry Jour. Bact., 1942, 43 573.

⁸³ Florey: Jour. Path. and Bact., 1933, 37.283

acute heart failure. His observations and conclusions have been confirmed by other workers (Drinker and Bronfenbrenner 1924, Grove 1932a, b).

Anaphylactic Shock in the Dog.—A sensitized dog, after receiving an intravenous injection of the specific antigen, shows marked signs of restlessness and excitement. Within a few minutes the animal vomits and usually passes urine and faeces, the latter sometimes blood-stained. It then collapses and lies prone. The muscles of the limbs are relaxed, and the dog appears extremely weak. Respiration is slow, and often deep and laboured. Stridor may be heard, and a little froth may exude from the lips. In rapidly fatal shock the weakness progresses, renewed diarrhoea and vomiting set in, and coma, occasionally associated with epileptiform convulsions, leads on to death. It has been pointed out by Dean and Webb (1924) that the reaction almost always occurs in two stages, the initial acute symptoms being followed by some signs of recovery. In fatal cases these soon give place to the second stage of increasing collapse. In other instances severe initial shock is followed by a rapid return to normal.

As demonstrated by Biedl and Kraus (1909), anaphylactic shock in the dog is associated with a progressive fall in blood pressure; later investigations (Pearce and Eisenbrey 1910, Dale 1920b) have made it clear that this is not due to any failure of output from the heart or to any influence of the central nervous system, but to the collection and stagnation of the blood somewhere in the tissues. The studies of Dale and his colleagues (Dale 1920b) indicate that capillary dilatation is the essential factor.

Manwaring (1910) performed a large number of experiments in an attempt to localize the site of reaction; and, having found that shock did not occur when the abdominal vessels were ligated, proceeded to explore the rôle of the various abdominal viscera by a series of operative experiments designed to exclude each in turn. He found that the exclusion of the liver inhibited acute shock in dogs; and his observations were amply confirmed by subsequent workers (Voegtlin and Bernheim 1911, Denecke 1914). These results accord well with the findings at necropsy. The congestion of the abdominal viscera has been noted by many observers; and Weil (1917) points out that in dogs dying of anaphylactic shock in its acutest form the single outstanding feature at necropsy is the enormous distension and congestion of the liver.

We may briefly note certain additional phenomena, which seem to be general to anaphylactic shock as displayed by any of the species so far investigated, though not developed to an equal degree in all of them. There is commonly a sharp decrease in the circulating leucocytes and platelets; and this, as Webb (1924) has shown, is associated with an aggregation of polymorphonuclear cells in the capillaries of the lungs, a phenomenon clearly analogous to that which follows the intravenous injection of bacteria in immunized animals. In rabbits, the arterioles of the ear are conspicuously contracted during shock; circulating leucocytes adhere to the capillary walls and in some cases form leucocytic emboli (Abell and Schenk 1938). Removal of circulating leucocytes and their retention in the tissues was also demonstrated by Dragstedt, Ramirez de Arellano and Lawton (1940) in the isolated lung of sensitized rabbits, when perfused with blood containing the specific antigen. The blood platelets share in the aggregation of leucocytes in the vessels of the lungs and other tissues. There is a decrease in the coagulability of the blood of the dog, but not of the blood of rabbits or guinea-pigs (see Adams 1953). The work of a number of investigators (see Dragstedt 1941) has excluded changes in the blood elements concerned in normal coagulation as the cause of this phenomenon, which appears to be due to the liberation of heparin during shock (Jaques and Waters 1940). There is a decrease in the amount of complement in the circulating blood (see Ecker 1939, Stavitsky *et al.* 1949); and there is a fall in the body temperature.

Anaphylactic Shock in the Mouse.—Mice are relatively resistant to shock. Refractoriness varies with the breed of animal, and in some cases depends on insusceptibility to sensitization, not insusceptibility to shock (Weiser *et al.* 1941). Large doses of antigen are required for sensitization, and for the intravenous shocking dose (Solotorovsky and Winsten 1953). Freund's adjuvant greatly improves the sensitizing power of proteia

perhaps, of the flushing action of the slightly acid urine. The few bacteria that may be present are confined to the region of the meatus. The normal vaginal secretion is acid and is markedly bactericidal toward most species of bacteria.

Normal Flora. A microorganism somewhat better able to resist the defensive mechanisms of the host but at the same time unable, except when resistance is reduced to a low level, to invade the body tissues, may exist in conjunction with the host as a part of the latter's "normal flora." The staphylococci which are able to resist the bactericidal action of the skin are almost invariably present on these surfaces and are regarded as "normal" inhabitants. The scanty bacterial flora of the vagina, on the other hand, is composed almost entirely of aciduric bacteria; and the bacterial types present in the intestines are determined to a considerable extent by the type of food material present, *i.e.*, the diet of the host, and by the pH of the various intestinal levels. The composition of the intestinal flora is also affected to an indeterminate extent by antagonistic relationships among the bacteria present and potentially present. It has been shown,⁸⁴ for example, that some strains of coliform bacteria elaborate an antibiotic, colicin, which is effective against dysentery bacilli. Certain kinds of bacteria, such as lactobacilli, spirochetes, various cocci and the like, exist in the mouth in the interstices between the teeth and in and under tooth plaques and constitute a normal flora characteristic of this region. The bacteria commonly present in the nose and throat consist of still different forms, such as pneumococci, Friedlander's bacillus, green and hemolytic streptococci, etc. These organisms, while in a sense a normal flora, are not so well established as the flora of some other regions, and the nature of the microorganisms present may be determined in large part by the kind of bacteria which are constantly entering the upper respiratory tract.

As long as the resistance of the host is maintained at a sufficiently high level, the bacteria constituting the normal flora do no harm. If, however, resistance is reduced in some manner, the more virulent forms may invade the tissues and set up an infection. The congestion of the nasal mucosa, and the consequent interference with ciliary activity and the movement of mucus, which follows the temperature shock of chilling, not infrequently make possible infection by bacteria such as hemolytic streptococci or pneumococci, which are already present.

⁸⁴Frederick and Levine: Jour. Bact., 1947, 54:785, Halbert. Jour. Immunol., 1948, 58:153.

animal that has received an injection of the serum of an actively sensitized animal will respond to the subsequent injection of the corresponding antigen by developing acute anaphylactic shock. Passive sensitization may be effected with equal or even greater certainty by an injection of the serum of an animal that has been "immunized" against a given antigen by repeated injections, so that it is not itself sensitive to the antigen in question. In either case the injection of the serum is not usually followed by the *immediate* sensitization of the recipient. An interval of some hours must elapse between the injection of the sensitizing serum and the injection of the corresponding antigen if typical shock is to be regularly obtained. For maximal sensitization the interval may be even longer—4 to 6 days (Kellaway and Cowell 1922). The incubation period is in part a function of the dose of antibody. In passive sensitization of guinea-pigs to a constant dose of ovalbumin, Benacerraf and Kabat (1949) induced fatal shock immediately after the antibody, but only when a dose of 2 mgm antibody nitrogen was used; whereas the injection of 0.06 mgm. antibody nitrogen sufficed for shock after a lapse of 5 hours.

No incubation period appears to be necessary for passive intravenous sensitization in the dog (Sherwood *et al.* 1948).

Under suitable experimental conditions, an analogous reaction, "reversed passive anaphylaxis," may be induced in the guinea-pig by injecting antigen first and antibody later (see Kellett 1935). Both in "reversed" and direct anaphylaxis, shock is produced when the two intravenous injections follow one another immediately (Dean *et al.* 1936, Zinsser and Enders 1936, van den Ende 1939). Van den Ende, testing serum proteins as antigens, obtained reversed passive anaphylaxis with globulin but not albumin—suggesting that the reaction is determined by the fixation of globulins—either as antigen or as globulin antibody.

The efficacy of an antiserum in the induction of passive anaphylaxis appears to be related to its content in specific antibody measured as precipitin (Doerr and Russ 1909). The amount of specifically precipitable antibody nitrogen required for passive sensitization of guinea-pigs to fatal shock was estimated by Kabat and Landow (1942) and Kabat and Boldt (1944) to be about 0.03 mgm.; that is, less than 0.2 mgm antibody globulin. Similar figures were obtained with both rabbit and guinea-pig antibody (Benacerraf and Kabat 1949). With this minimal sensitizing dose, the optimal shocking dose of ovalbumin was about 50 times the amount serologically equivalent (Chapter 7) to the antibody injected. It should be noted that non-precipitating antibody is also effective in passive sensitization (Kabat and Benacerraf 1949). This type of antibody, discovered first as a co-precipitating antibody (p. 235), must not be confused with the non-precipitating, skin-sensitizing antibody discussed on p. 1321, which does not necessarily confer anaphylactic hypersensitivity. There seems no reason for supposing that the reagents concerned differ from those with which our other immunological studies have made us familiar. The experimental study of this phenomenon has, however, brought to light a relationship that is probably of fundamental importance in immunological mechanisms. Different antisera, produced by the immunization, or sensitization, of animals of different species against a single antigen, differ widely in their ability to induce passive sensitization in a given species of animal.

Thus, Avery and Tillett (1929) and Brown (1934b) found that guinea-pigs could be sensitized to pneumococcal polysaccharides by the injection of antipneumococcal sera prepared in the rabbit, but not by antipneumococcal sera prepared in the horse. Again (see Friedberger and Hartoch 1909, Scott 1931, Hartley 1940), guinea-pigs cannot be

Chapter 9

THE TRANSMISSION OF INFECTION

Whatever the pathogenic powers of a bacterium and the efficiency of the defensive mechanisms of the host, an essential preliminary to the production of infectious disease is a meeting of the parasite and its prospective host. In some instances in which the bacterium is naturally saprophytic, it enters the body by accident, so to speak; such, for example, appears to be the case in tetanus, gas gangrene and similar infections. In most instances, however, the bacteria that produce disease are more or less closely adapted to a parasitic existence, and pass from one animal body to another with only a relatively brief sojourn in the external world. In general, then, the transmission of infection is a process in which the causative microorganism is transferred, either directly or indirectly, from a diseased to a healthy susceptible animal.

The elucidation of the mechanisms involved in this transfer is a matter of considerable practical as well as theoretical importance. If the sequence of events that precedes infection is known, it may be, and often is, possible to interrupt it at its most vulnerable point and thereby control the spread of disease. From the theoretical point of view, disease is by no means entirely a matter of host resistance and microbic virulence; it is, in a very real sense, the outcome of the interaction of the host and parasite populations. It is at this point that the study of the infectious diseases transcends the bacteriology of clinical medicine with its emphasis on the individual case, and assumes broad biological significance as a problem in interspecies competition.

The equilibrium that tends to become established between the host and parasite populations is an unstable one in that the factors which determine it—i.e., the character of the host population in particular and possibly that of the parasite population, as well as the environmental factors which affect their relationship—are constantly shifting, and the equilibrium ever has a tendency to establish itself at a new level. The shift may be a sudden and violent one whose outward manifestation is an explosive outbreak of disease or, less commonly, may take the form of a gradual increase or decrease in the incidence of the disease.

The factors associated with the maintenance or shift of this equilibrium are the subject matter of *epidemiology*.¹ The term epidemiology is best regarded in this broad sense and therefore includes the study of the transmission of endemic disease, i.e., disease which has a low incidence but is con-

¹ For a general discussion of the principles and methods of epidemiology see Frost: *Collected Papers*, pp. 493-542. Commonwealth Fund, New York. 1941. A brief discussion of theoretical epidemiology is given by Aycock and Russell. *Amer. Jour. Med. Sci.*, 1943, 206-399.

or galactoside did not induce shock when injected into sensitized animals, but each inhibited anaphylactic shock when injected immediately prior to the injection of the corresponding carbohydrate-protein compound. Klopstock and Selter (1929) carried out similar experiments and obtained similar results, using compounds of protein with atoxyl, or with the sodium salt of metanilic acid. Landsteiner and Levine (1930) sensitized guinea-pigs to the D- and L-isomers of *p*-amino-tartranilic acid, in each case coupled to horse globulin. The subsequent injection of the corresponding active groupings coupled to chicken globulin induced anaphylactic shock. The shock could be specifically inhibited by a preliminary injection of a compound of resorcinol with the active grouping concerned. Similar results were obtained with guinea-pigs passively sensitized to *p*-amino-arsanilic acid, the active substance being coupled to tyrosine for the inhibition tests. In later experiments Landsteiner and van der Scheer (1932, 1933, 1938) were able to induce shock by injection of the azo-dye compounds alone into animals sensitized with the appropriate azo-dye-proteins.

Specific Desensitization.—The specific desensitization of sensitized animals was recorded by many of the earlier investigators (Otto 1905, Rosenau and Anderson 1906, 1907, 1908, Besredka and Steinhardt 1907, Gay and Southard 1908). Desensitization—as then observed, and in the sense in which this term is now commonly employed—differs from the inhibitory effect induced by simple haptens in that the desensitizing agent is the complete antigen, which is capable, when administered intravenously in adequate doses, of inducing typical shock. The desensitizing effect is obtained, in place of acute shock, if a very minute dose of antigen is given and repeated, or if rather larger doses are given by a route, such as the subcutaneous, which ensures slow absorption. The essential conditions for success appear to be the administration of an amount of antigen that is adequate in total by some method that prevents any rapid accumulation in the circulating blood. It must be emphasized that desensitization, however it is induced, is purely temporary, the anaphylactic, or hypersensitive state being re-established in a few days or weeks (Vallery-Radot *et al.* 1936, Adelsberger 1936, Gernez 1936). There is no good evidence of a permanent removal of an allergic state by specific desensitization.

The Mechanism of Anaphylactic Shock.

It is clear that acute anaphylactic shock, as induced by any of the procedures that have been described above, belongs to the category of the specific antigen-antibody reactions. The only question at issue is the mechanism by which this primary reaction gives rise to the series of events that characterize acute shock in any given animal species.

There are two opposing views. According to one the reaction between antigen and antibody, occurring in the circulating blood or in the tissue fluids, leads to the production of a toxic substance, anaphylatoxin, which, acting on susceptible cells, gives rise to the characteristic syndrome. According to the other the primary reaction occurs not in the blood or tissue fluids but on or in the tissue cells; and the reactions that characterize acute shock are due not to any toxicity of the antigen-antibody compound itself, or of any derivative from it, but to cellular disturbances initiated by the antigen-antibody reaction. As we shall see, the picture that has been pieced together during the last twenty years or so derives, in all its essential outlines, from the cellular school, though it contains details reminiscent of the anaphylatoxin hypothesis.

The Humoral or Anaphylatoxin Hypothesis.—The chief protagonist of this conception of the mechanism of acute anaphylactic shock was Friedberger (1910, 1912,

stantly present in a population, as well as the study of *epidemic* disease, i.e., disease of high morbidity which is only irregularly present in clinically recognizable form. In this connection it may be noted that the term *pandemic* is often applied to an epidemic of unusually great proportions. These categories, although useful, are not mutually exclusive; a disease endemic in a community may, at times, attain the proportions of an epidemic and later subside to an endemic level.

Essential to knowledge of the epidemiology of disease are certain characteristics of the etiologic agent and of the clinical infection which determine the possible channels of transmission. The more important of these are.

- (1) the route by which the infective agent enters the body,
- (2) the route by which the infective agent leaves the body,
- (3) the resistance of the microorganism to the deleterious effects of the outside environment;
- (4) presence or absence of an intermediate host; and
- (5) the relation between frank, clinically recognizable disease and the discharge of virulent bacteria from the body.

The Carrier. With regard to the last, additional explanation is desirable. In the early days of bacteriology it was assumed that the contact of host and parasite could have only one or the other of two outcomes; either no infection occurred, owing presumably to high resistance on the part of the host, or clinically characteristic disease developed in the individual. More recently it has become clear that an intermediate state, the establishing of a symptomless infection, may occur. Such infections are, of course, inapparent and concealed and may be demonstrated only by isolation and identification of the infectious agent. An individual so infected is termed a carrier.

Two types of carriers are commonly differentiated, the casual carrier who harbors the microorganism temporarily, a matter of a few days or weeks, and the chronic carrier who remains infected for a relatively long time, sometimes throughout life. Such individuals serve, of course, to disseminate the infectious agent. In the first group are the great majority of carriers of the diphtheria bacillus, meningococcus, pneumococcus, certain streptococci, etc., and the second includes carriers of certain of the enteric bacilli, especially the typhoid bacillus. A third type of carrier is often differentiated, the convalescent carrier who remains infected for a greater or lesser length of time after recovery from the disease. These last do not, of course, fall into the category of concealed infections. Sharp separation is sometimes not possible, however, for the casual or chronic carrier may, in fact, be convalescent from the disease in a form either atypical or so mild as to go unrecognized (ambulatory cases).

While it is now commonplace to recognize the existence of the carrier state, the implications of the general principle that infection may occur without disease are frequently neglected. Thus it follows that clinically apparent infections may constitute only a part, in some instances only a very small part, of the infections continually taking place. Clearly, then, if an important proportion of the infections are of the concealed type, a reasonably accurate estimate of the extent to which the infection is disseminated in the host population cannot be arrived at on the basis of cases of the disease. The implications

anaphylactic reactions in the guinea-pig—is in general a prominent feature, though it may vary in degree. There is, however, a high frequency, with these anaphylactoid reagents, of capillary thromboses and embolism in the lungs.

Among such anaphylactoid reagents—and this has been a source of error in several experiments supposed to support the humoral view—must be included all antisera that contain a high concentration of Forssman antibody (see Doerr and Pick 1913, Taniguchi 1922, Scott 1931; see also Neter 1947). The toxic effect is here due, in part at least, to a direct action of the heterophile antibody on the corresponding antigen contained in the guinea-pig's tissues, and may be regarded as a peculiar example of reversed passive anaphylaxis. When the serum is injected by the ordinary intravenous route the first stress of this reaction falls on the endothelium of the lung capillaries, resulting in lesions which appear to be identical with those produced by the scrotoxins.

Another anaphylactoid reagent is ordinary peptone (de Waele 1907, Biedl and Kraus 1909, Hirschfelder 1910, Doerr 1914, 1922, Dale 1920a, 1929, Manwaring, Clark and Chilcote 1923). The resemblance of peptone shock to true anaphylactic shock is very striking. In the guinea-pig there is the typical contraction of the bronchioles, in the dog the fall in blood pressure and the congestion of the liver. In both there may be found the loss of coagulability of the blood and the multiple small subserous hæmorrhages that are characteristic features of acute anaphylaxis in general. In dogs peptone shock is apparently accompanied by a release into the blood of heparin and histamine (Quick 1936, Holmes *et al.* 1941, Rocha e Silva *et al.* 1947); and peptone activates proteases in the serum (Burdon *et al.* 1951, Ungar *et al.* 1953). Peptone and other anaphylactoid agents cause aggregation of leucocytes in the blood vessels of the lung and other organs, as occurs also in anaphylactic shock. After peptone shock, as after non-fatal anaphylactic shock, dogs remain in a refractory state, but peptone refractoriness depends on the degree of shock suffered, and cannot be induced by sub-shocking doses of peptone (Dragstedt 1913a).

Histamine Shock.—Histamine is an amine derived by decarboxylation from histidine. The toxic syndrome produced by histamine reproduces very faithfully several of the characteristic features of acute anaphylaxis. In the guinea-pig the spasm of the bronchioles is constantly present in its most typical form. In the dog (Maunter and Pick 1915, Dale 1929) the muscular walls of the efferent hepatic veins are very sensitive to histamine; in response to traces of this substance they constrict and dam back the blood in the capillary spaces of the liver. But there are also quite definite points of difference. The distension of the dog's liver with blood and lymph in anaphylaxis is far more severe and persistent than that produced by histamine. Nor does the blood become incoagulable in histamine poisoning; but this we should expect if the incoagulability was due to the liberation of heparin.

When powerful histamine-liberating drugs (see Feldberg and Miles 1952) are injected intravenously into guinea-pigs, the resulting release of histamine from depots of bound histamine in the body produces an acute anaphylactoid shock.

The importance of these observations lies in the fact that histamine is a relatively simple substance of known chemical constitution, the pharmacological action of which has been studied in considerable detail. It can hardly be a mere coincidence that on intravenous injection it mimics so closely the syndrome produced by the injection of a non-toxic antigen into a sensitized animal.

Summarizing the evidence up to this point, we recognize a number of substances, ranging in complexity from histamine and synthetic histamine-liberators to enzymic proteins, which can induce a shock mimicking anaphylactic shock. With some of these, the shock is produced by the liberation of histamine; with others, it is associated with the activation of serum proteases. We shall defer the discussion

of this are several. For example, diseases which occur sporadically and do not seem to be easily or often transmitted from the sick to the well, such as poliomyelitis, meningococcus meningitis, lethargic encephalitis, pneumococcus pneumonia, etc., may be as widely disseminated and readily communicable as measles or the common cold, but the clinically distinctive disease is the exception rather than the rule. Furthermore, the rise or decline of infectious disease or its age or geographical distribution may not reflect a corresponding variability in the prevalence of the infection but may, rather, be a consequence of variation in the case-carrier ratios. Though here inferred from the observed occurrence of the carrier state, none of these possibilities remains purely hypothetical for all have been found to exist. Thus, carriers of virulent pneumococci do not occur predominantly in the higher age groups, nor diphtheria bacillus carriers in the school child, where the morbidity of these diseases is highest; diphtheria bacillus carriers are as common in the tropics as in temperate climates despite the relative rarity of clinical diphtheria in the hot climates, and so on. In many other cases, such as that of poliomyelitis, such a situation is suspected but technically difficult to prove. It will be obvious, therefore, that the recognition of the carrier state and its implications is basic to sound epidemiological thinking and of primary importance to the understanding of the mechanism of spread of the infectious diseases.

The epidemiological factors given above are most readily and most satisfactorily determined by experimental study when a disease is of known etiology, but sometimes may be approximated to a relatively satisfactory degree by indirect evidence. For example, although the famous Broad Street Pump epidemic occurred prior to the discovery of the etiology of cholera, the indirect evidence plainly indicated to Snow that the infective agent left the body in the feces and entered the gastro-intestinal tract via the contaminated well water.²

Epidemiological Types of Infectious Disease. On the basis of such fundamental information, the infectious diseases may be separated into a number of epidemiological types which, despite certain limitations, serve to illustrate the diversity of ways in which infection may be disseminated.³ A rough classification, based on the assumption that the human being is the recipient of infection and that the control of diseases of man is the point at issue, follows:

- (1) diseases of lower animals transmissible directly to man (rabies, tularemia, glanders, etc.);
- (2) diseases of animals or man transmitted by insect vectors in which
 - (a) the insect acts as a mechanical carrier (the house fly and typhoid fever),
 - (b) the parasite multiplies in the insect vector (bubonic plague),
 - (c) the parasite is transmitted from one insect generation to the next by egg infection (spotted fever), and
 - (d) the parasite undergoes a portion of its life cycle in the insect (malaria);
- (3) diseases of animals or man transmitted indirectly
 - (a) by water (the enteric infections such as typhoid fever, cholera, etc.),
 - (b) by milk (scarlet fever, bovine tuberculosis, undulant fever, etc.).

² The original papers have been reprinted under the title *Snow on Cholera*. Commonwealth Fund, New York. 1936.

³ Epidemiological types of infectious disease are discussed briefly by Baker: *Amer. Jour. Trop. Med.*, 1943, 23 559.

be rendered hypersensitive by prolonged perfusion with diluted serum from anaphylactic or immunized guinea-pigs. This hypersensitiveness is hardly perceptible after 1 hour's perfusion, but it is easily perceptible after 5 hours' perfusion, a period strikingly similar to the latent period in passive sensitization in the living animal. Nevertheless, an almost immediate sensitization of the isolated uterus can be produced *in vitro*, resembling in speed that which must precede the anaphylaxis following the almost simultaneous injection of antigen and antibody into the whole animal.

A fact of great significance was established by Dale while investigating the response of the uteri from immunized guinea-pigs. These animals had received repeated injections of horse serum increasing in amount. Some of them were injected intraperitoneally with 5 ml. of horse serum but showed no sign of shock. Nevertheless, the uterine horns, removed from other animals of the same series, reacted with a typical contraction on the addition of horse serum to the bath, thus showing that fixed antibody is present in the immune as well as in the hypersensitive animal, and lending strong support to the view that the immune guinea-pig is protected by the excess of antibody in its circulating blood.

The protective function of circulating antibody is clearly illustrated in some of Weil's experiments (see Fig. 270). A series of guinea-pigs were passively sensitized by the intraperitoneal injection of 0.1 ml. of rabbit-v-horse serum, i.e. the serum from a rabbit immunized by repeated injections of horse serum. Three days later one animal received 2 ml. of this antiserum (20 sensitizing doses) and immediately thereafter 0.01 ml. of horse serum (1-2 anaphylactic doses). This guinea-pig showed slight symptoms only. It was killed; its uterus was tested by Dale's technique, and its serum was injected into another normal guinea-pig which was tested 24 hours later by the injection of horse serum. The uterus of the first animal gave a typical reaction, showing that it had anchored the antibody injected 3 days previously, and that this fixed antibody had not combined to any appreciable extent with the 0.01 ml. of antigen subsequently injected under cover of a protective dose of antibody. The second guinea-pig developed typical shock, showing that a considerable amount of the antibody injected into the first guinea-pig immediately prior to the injection of the antigen had remained unneutralized in the circulation. Another sensitized guinea-pig of the same series received 2.5 ml. of the rabbit-v-horse serum (25 sensitizing doses) and immediately thereafter 0.4 ml. of horse serum (52 anaphylactic doses). It died in acute shock, showing that the amount of antibody present was not sufficient to protect the cells containing the fixed antibody from this excessive dose of antigen. The uterus, removed immediately after death, was injected

serum. It developed moderate symptoms only, showing that the greater part of the circulating antibody in the animal that died of acute shock had been neutralized by the large dose of antigen. It should be noted, however, that serum antibody does not always protect. Morris (1936a) recorded that an excess of antibody injected into sensitized guinea-pigs led to an increase, not a decrease, of sensitivity.

Using antigens labelled with radioactive iodine as shocking agents, Dixon and Warren (1950) observed an apparently specific uptake of antigen by the lung of the anaphylactic guinea-pig.

Taking the evidence as a whole, the different behaviour of the hypersensitive and the "immune" guinea-pig may with some confidence be attributed to a difference in the relative concentration of fixed and circulating antibody.

If, however, we turn to other animals the evidence is more confused (see Dale 1920b, Scott 1931); and the observations of Manwaring and his colleagues (1923-28) on acute anaphylaxis in the dog are particularly difficult to fit into the relatively simple picture that we have drawn above. It seems, however, altogether unlikely that the mechanism of acute anaphylaxis differs fundamentally from one animal to another; and it is probable

- (c) by food (typhoid and paratyphoid fevers), and
- (d) by inanimate objects or fomites, such as books, towels, etc. (scarlet fever, diphtheria and the like),
- (4) diseases of man transmitted directly
 - (a) by infective droplets—air-borne infection (the respiratory diseases and others) and
 - (b) by direct contact (the respiratory and venereal diseases in particular).

Air-Borne Infection. Of these modes of transmission air-borne infection is one of the most important. In recent years Wells⁴ and his associates have shed new light on the mechanism of transmission of disease, especially respiratory disease, from man to man by the experimental elucidation of droplet infection. It was postulated many years ago by Pflügge that such diseases could be transmitted by infective droplets. The droplets which he studied, however, were greater than 0.1 mm. in diameter and fell to the ground soon after expulsion by sneezing or coughing, seeding the air for only negligible distances. Epidemiological considerations, however, seemed to demand air-borne infection effective at considerable distances. Wells and his co-workers have shown that Pflügge's evidence was incomplete and that under the usual conditions of humidity particles or droplets smaller than 0.1 mm. in diameter are evaporated completely before reaching the ground, leaving suspended nuclei consisting essentially of organic matter, salts and bacteria, to become, for all practical purposes, a part of the atmosphere. The survival of pathogenic microorganisms in such nuclei is, of course, largely a matter of their resistance to drying, and it has been shown that some of the respiratory pathogens, including viruses such as that of influenza, may remain viable and infective for many hours under these circumstances.⁵ The process of expulsion of such droplets in coughing, sneezing and talking has been photographed by Jennison⁶ and is illustrated in Fig. 30. Studies of the air in various types of rooms under various conditions have yielded data on the quantitative aspects of air contamination, green streptococci have been used as indicators of air pollution in much the same manner as *Bacterium coli* is used for studies on water pollution. In general, enormous numbers of bacteria of respiratory origin have been found in the air of crowded rooms, especially when the occupants are sneezing, and there can be no doubt of the significance of these observations in relation to the explosive spread of respiratory disease in a non-immune population.

Evidence has accumulated in recent years⁷ which strongly suggests that the inhalation of air-borne bacteria in dust may be an even more important factor in the dissemination of infectious disease of the respiratory tract than that of directly expelled infected droplets. The use of oiled blankets and floors under experimental conditions in hospital wards has, in a number of instances, proved a highly efficacious method of control of the spread of infection. The direct destruction of air-borne bacteria by the use of glycol and

⁴ See the review articles by Wells: *Jour. Amer. Med. Assn.*, 1936, 107:1698; and by Wells and Wells: *Amer. Jour. Med. Sci.*, 1913, 206 11.

⁵ Cf. the review by Buchbinder. *Jour. Amer. Med. Assn.*, 1942, 118 718.

⁶ Jennison *Amer. Assn. Advancement Sci.*, Pub. No. 17, 1942, p. 106.

⁷ For a discussion see *Amer. Jour. Pub. Health*, 1948, 38 409

The Rôle of Histamine in Anaphylaxis.—The evidence for the essential rôle of histamine in anaphylactic shock falls under four heads. Firstly, the likeness between histamine shock and anaphylactic shock, which we have already noted; secondly, the demonstration that tissues contain histamine that can be mobilized by suitable stimuli; thirdly, the demonstration of histamine mobilization during shock; and fourthly, the suppression of shock by measures that destroy or inhibit histamine or inhibit the reaction.

Lewis (1927), in his classical studies on the reaction of the skin to a localized stimulus or injury, was able to show that the local reddening, due to the dilatation of the minute blood vessels, and the succeeding local wheal, were independent of the local neuro-vascular mechanism, whereas the surrounding flare, due to the dilatation of the neighbouring arterioles, was dependent on a local axon reflex. He concluded that the reddening and the wheal formation were the result of a chemical stimulus provided by some substance liberated from the injured cells; and he found that histamine, alone among the many known substances that he tested, reproduced in complete detail the vascular changes, including increased capillary permeability, induced by the hypothetical cell constituent, which he referred to as the *H-substance*. Dale and his colleagues (see Dale 1929, Dale, Dudley and Thorpe 1927, Harris 1927, Thorpe 1928) showed that histamine is a normal constituent of many different tissues; and the evidence available strongly supports the view that it exists during life in an indiffusible form, from which it can be released without gross damage to, or death of, the cells that contain it.

The relative histamine content of different tissues is interesting and suggestive. Using a physiological method of assay (see Thorpe 1928, Harris 1927) the lungs have been found to contain between 35 and 75 mgm. of available histamine per kilo, the liver between 2.5 and 6.6 mgm., the skin as a whole about 6 mgm. (2.4 mgm. in the epidermis and 4 mgm. in the dermis). Other tissues contain much less. The voluntary muscles contain about 1 mgm. per kilo, the testis about 1.8 mgm., the submaxillary gland about 0.5 mgm., and the thyroid about the same. These figures are mainly for the organs of horse and ox. The histamine content of the different organs varies with the species of animal. Thus the skin of the rat contains 30–40 mgm./kg., but that of the guinea-pig only about 6 mgm./kg. Again, the blood of rabbits contains 1 to 5 μ g./ml., that of man and the guinea-pig less, and that of the dog very little. The blood histamine, it should be noted, is mainly in the granulocytes and platelets (see Code 1952). The plasma normally contains very little; free histamine is fairly rapidly destroyed by histaminase or diamine oxidase in the plasma.

after
in the blood, they calculated the amount present in the animals at the onset of shock and found it enough to account for the vascular reactions of the shocked animal. The substance was specifically destroyed by the enzyme histaminase (Dragstedt and Mead 1936b; see also Code 1939).

Watanabe (1931) obtained some evidence that the histamine content of the lungs of sensitized guinea-pigs was abnormally high, and much less in the lungs of animals after shock. Bartosch, Feldberg and Nagel (1932) were the first to demonstrate a histamine-like substance in the perfusate of sensitized guinea-pig lungs when antigen was added to the perfusion fluid. This observation was confirmed by a number of workers, and the identity of the substance with histamine more firmly established (see Schild 1939). Increased quantities of the histamine-like substance were also found in the blood of the whole guinea-pig after shock (Simons and Staub 1937, Code 1939). Large quantities of histamine are liberated from the liver of sensitized dogs, and heparin into the perfusate. Release is better when unclothed blood is used.

other bactericidal aerosols (p. 150) and ultraviolet irradiation is not only feasible in some circumstances but has given encouraging results.



Fig. 30 The atomization of mouth and nose secretions demonstrated by high speed photography. 1, A violent sneeze in a normal subject, note the close approximation of the teeth, resulting in effective atomization. 2, Head cold sneeze, note the strings of mucus and the less effective atomization of the viscous secretions 3, A stifled sneeze. 4, Sneezing through a dense face mask. 5, Cough, note the lesser discharge than in the uninhibited sneeze. 6, Enunciation of the letter f. (Jennison.)

Even though air-borne infection, whether by infected droplets or dust-borne bacteria, is an important factor in the spread of respiratory disease, very often direct contact with infected persons may assume a major role.

As may be inferred from the outline above, a disease not infrequently has

even how far we should locate the injury and liberation of histamine in one type of cell and the response to the liberated histamine in another. We have noted discrepancies between the relative histamine content of different organs and the damage they suffer in shock. There are other hints of a distinction between the source and the site of action of the liberated histamine; for example, isolated plain muscle from the sensitized dog gives no significant response to the specific antigen, but the plain muscle of the dog is sensitive to histamine.

We may now consider briefly the effect of anti-histamine substances on anaphylactic shock. Histamine is readily destroyed by the enzyme histaminase (Best and McHenry 1930). However, though Karady and Browne (1939) claimed to have protected sensitized guinea-pigs from shock by intravenous injections of the enzyme 15 minutes before the shocking dose, their findings were not subsequently confirmed (Knoll 1940, Rose and Browne 1941, Youngner, Freedman and Nungester 1941, Alexander and Bottom 1940).

A large number of substances have now been synthesized which inhibit the pharmacological action of histamine, some more specifically than others. Most of the more effective of these drugs diminish, and may even suppress, the symptoms of anaphylaxis in the experimental animal which are referable to free histamine. They do not affect sensitization; nor do they appear directly to affect the antigen, the antibody, or the antigen-antibody combination; and they act by neutralizing the released histamine. Much larger doses are usually required than those necessary to antagonize the estimated amounts of histamine released (see the review of Feinberg *et al.* 1950).

The administration of sublethal doses of histamine induces a state of increased tolerance to the drug. Farmer (1939) and Karady (1941) contend that histamine desensitization is accompanied by a moderate degree of desensitization to specific anaphylactic shock. Tell and others (1943, Rodney and Fell 1943) immunized rabbits with horse globulin coupled with the pharmacologically inactive *p*-aminobenzoyl histamine, and induced the formation of antibodies whose specificity was partly determined by the histamine. The immunized animals were then sensitized with ovalbumin. As compared with non-immune sensitized controls, there was definite evidence of a diminished liberation of histamine into the blood during shock, and of a diminished skin sensitiveness either to shocking doses of antigen, or to histamine. Immunized guinea-pigs, subsequently sensitized to ovalbumin, were largely protected against fatal shock. Coffin and Kabat (1946) confirmed this result, but found the effect to be non-specific, because immunization with unmodified horse protein was also protective.

The Rôle of Acetylcholine and other Endogenous Substances in Anaphylaxis.—Went and Lissak (1936, 1938) and Martin and Went (1939) reported that, during the perfusion of the heart of a sensitized guinea-pig with antigen solution, a substance resembling choline is liberated from the muscle, and that the heart muscle of sensitized guinea-pigs is more sensitive than the normal heart to the pharmacological action of choline. Went suggested that choline and acetylcholine, as well as histamine, may be liberated from different organs during shock. Wenner and Buhrmester (1937) found acetylcholine in the blood stream of rabbits after shock, but Ratnof (1939) could not confirm their findings. Kourilsky and his colleagues (1938) detected no acetylcholine in the hæmorrhagic lesions of shocked animals. They noted that acetylcholine produced no hæmorrhages in the tissues on local injection, whereas histamine did so. Nakamura and his colleagues pointed out that acetylcholine shock and anaphylactic shock have many features in common, but few in common with histamine shock, both in symptomatology and modification by physostigmine, eserine and other drugs. They attributed the main symptoms of anaphylactic shock to acetylcholine liberated as the result of antigen-antibody union in the region of the nerve-endings of the sympathetic system (Nakamura and Takahashi 1938, Nakamura 1941, Chigira 1941). Farber, Pope and Landsteiner (1944) were able regularly to demonstrate histamine in shocked guinea-pig lung; only in shocked heart

a certain epidemiological character which may at times be even more familiar to the epidemiologist than the causative agent is to the laboratory worker. In the case of epidemic influenza, for example, the disease is, at present, an epidemiological rather than clinical entity and is diagnosed with reasonable accuracy only during epidemic periods. Similarly, epidemiological studies have shown the existence of two kinds of smallpox, the one a relatively innocuous variety with a low case fatality and the other a severe disease (so-called malignant or "black" smallpox) with a high case fatality. The difference between these varieties is real and of no small practical importance even though indistinguishable by clinical observation of the individual case (p. 859).

The epidemiology characteristic of a given disease shows, on the one hand, certain, and sometimes close, relationships to other similar infections, and, on the other, a certain variability which arises as a result of transmission in more than one way. The enteric infections, cholera, typhoid fever and the bacillary dysenteries, are similar to one another in epidemiology but are quite different in this respect from the respiratory diseases or the insect-borne diseases. The epidemiology of typhoid fever, however, is variable within limits and depends to some degree upon whether the disease is water-borne, milk-borne or transmitted by food or contact. Clearly, then, although it is possible to speak of certain broad principles of epidemiology, the epidemiology of a disease is in itself a special case and must necessarily be considered elsewhere (see later chapters) under the head of that disease.

The epidemiological character of a disease is dependent primarily upon certain aspects of the microorganism and the clinical infection as indicated above, and secondarily upon the habits, environment and mass susceptibility of the host population. To return to the example of typhoid fever: any mode of transmission is necessarily one that provides a connecting link between infectious fecal matter and the mouth of a susceptible human being, and the possibilities are, to this extent, limited. The age, sex and seasonal incidence, to some extent the case fatality, and the geographical and sociological distribution of cases are reflections of both the mode of transmission and the character of the population. In the case of water-borne typhoid, the drinking water supplies the connecting link, and the ensuing epidemic, limited to the area supplied by the contaminated water, shows no respect for age, sex or economic status. Milk-borne typhoid, on the other hand, geographically limited by the route by which the infected milk is delivered, exhibits an increased incidence in the lower age groups and among females, and is somewhat more frequent among those of higher economic status. Other modes of transmission are similarly reflected as minor variations in the epidemiological character of the disease.

Although the characteristics of the infectious agent and the clinical disease determine the *means* by which disease may be transmitted, the *extent* of its spread is a mass phenomenon determined by the character of the host and parasite populations and, as a corollary, their interaction with one another.

The Bacterial Population. The well-known variation in the severity and "contagiousness" of the infectious diseases is a consequence of corresponding variation from one species of pathogenic bacteria to another in

of the earlier injections. The rabbit, as a whole, had become so sensitized that it reacted in this characteristic and abnormal way to the injection of the specific antigen at any site (see also Seegal, Seegal and Jost 1932, Davidoff, Seegal and Seegal 1932). This reaction, in its typical form, appears to be peculiar to the rabbit. However, similar local changes occur on injection of antigen into species like the guinea-pig, rat and dog, provided that an adequate amount of precipitating antibody is present in the circulation. The experimental preference for the rabbit is probably based upon the greater readiness with which this species forms suitable antibodies. There is intense inflammation at the site, characterized by adherence of polymorphonuclear leucocytes to the vessel walls, followed by migration into the surrounding tissues and the development of mural thrombi of leucocytes and platelets, and some necrosis of the endothelium of the arterioles and venules. After about 24 hours the polymorphonuclear response is gradually replaced by a less intense mononuclear cell infiltration, and a process of repair begins, accompanied by the development of multiple discrete foci of plasma cells (Gell and Hinde 1954).

Abell and Schenk (1938), by means of a transparent window grafted into rabbits' ears, observed that half an hour after introduction of the antigen there was an intermittent segmental spasm of the arterioles, which was soon followed by the histological changes outlined above. The cause of the arteriolar spasm and adherence of granulocytes to the vascular endothelium is not known, but when the invasion of the tissues by circulating granulocytes is prevented, much of the œdema and vascular damage characteristically seen in the reaction is eliminated (Stetson 1951; and J. H. Humphrey, unpublished).

The Arthus reaction appears therefore to have at least three components: (a) relatively rapid events whose visible effect is upon the local blood vessels but which do not by themselves cause extensive necrosis; (b) the formation of leucocyte-platelet thrombi and intense invasion of the tissues by polymorphonuclear leucocytes, which is accompanied by, and may actually be the cause of, œdema and necrosis of the vessel walls; and (c) a delayed infiltration by macrophages and histiocytes, which probably reflects the response of the body to a foreign protein localized by the allergic reaction, and may even lead eventually to antibody production at the site. It is possible, however, that this last stage represents a "tuberculin-type" of reaction (see p. 1329) due to the simultaneous existence of two types of hypersensitivity in the actively immunized animal.

The Arthus Phenomenon; Passive Sensitization.—The capacity to produce an Arthus reaction can be passively transferred by injection of precipitating antibody from the same or another species, and the intensity of the reaction produced is proportional to the amount of precipitin in the tissues (Culbertson 1935). The studies of Kabat and his colleagues (1947, Benacerraf and Kabat 1950) showed that, in the rabbit and guinea-pig, the reaction occurred when either the antibody or the antigen was introduced into the body first, and that the intensity of the reaction was proportional to the amount of antibody present, within a wide range of antigen concentrations, provided the amount of antigen was in excess of that required to neutralize the antibody. Minimal reactions were produced in the rabbit when 25 μ g. of antibody N were injected intracutaneously, followed half an hour later by antigen either intravenously or into the same site. In the guinea-pig, intravenous injection of 0.41 μ g. antibody N was sufficient to produce severe reactions, and of 0.09 μ g. to produce mild reactions, when followed by antigen intracutaneously half an hour later. These amounts are much larger than those required for sensitization to "immediate" anaphylactic reactivity (p. 1302). Unlike passive anaphylaxis, the capacity

their ability to invade the body tissues and, once established, to produce clinical disease. As indicated in a previous chapter, a single species is potentially variable in these respects, for such variation can be induced by appropriate experimental manipulation. The possibility of such intraspecies variation in a bacterial population existing under natural conditions is one that has intrigued students of infectious disease for many years.

It is tempting to account for the genesis and rise of epidemic disease by assuming that the causative agent of a disease of endemic proportions gains in virulence by successive passage from person to person until its pathogenicity is so enhanced that an epidemic ensues. Similarly, a sojourn in a host population containing an increasingly large proportion of immunes might be expected to result in a diminution in the virulence of the microorganism and consequent subsidence of the epidemic. Furthermore, successive epidemic waves might conceivably result from periodic fluctuations in the virulence of the parasite. Unfortunately for such an explanation, there is little or no direct evidence that alterations in virulence play an important part in the evolution of single or secondary epidemic waves, and, in nature, bacterial virulence appears to be a relatively stable character.*

On the other hand, differences in the severity of a single disease from one epidemic to another are, in part, attributable to the existence of strains of the infectious agent which differ from one another in virulence. Benign and malignant smallpox referred to above is a case in point, and some workers believe that some strains of the diphtheria bacillus produce a more severe disease, (i.e., with a higher case fatality) than others (p. 611). Although variable from one strain to another, available evidence indicates that within a single strain virulence does not fluctuate to a demonstrable degree.

Possibly attributable in part to alterations in bacterial virulence are the changes in morbidity and mortality of some diseases such as scarlet fever, syphilis and tuberculosis, over long periods of time. In the case of scarlet fever the twenty-five years prior to 1830 was a period of very low death rates and was followed by a forty-year period of high death rates. Since then the death rate has declined and, though the incidence remains high, the case fatality is relatively low. In other diseases only a decline has been observed. Syphilis is no longer the scourge it was in the sixteenth century, and the present decline in tuberculosis began before the institution of preventive and therapeutic measures. In still other diseases, such as measles, no such long-term alterations in prevalence have been observed. Information is as yet too limited to assess these phenomena; possibly in some diseases there are long-term periodic fluctuations in bacterial virulence (this may be an artifact and represent only variations in the prevalence of virulent "epidemic" strains of the parasite) while in others an adaptive reduction in virulence or increase in resistance on the part of the host or a combination of both may play a part.

In general, it may be said that, in the short view, the bacterial population, as it exists in nature, is remarkably stable in so far as its ability to produce disease in a host population is concerned. Although the severity of a disease

* Cf. Aycock, Lutman and Foley *Amer. Jour. Med. Sci.*, 1945, 209:395.

been given the name of "serum sickness." An excellent description of the disease and a detailed discussion of its significance are contained in the monograph of von Pirquet and Schick (1905), and in the review of Longcope (1943). The great extension of serum therapy in modern times has provided the data for large-scale surveys of the incidence and kinds of serum sickness (see Park 1921, 1928, Beer 1938, Lyall and Murdick 1938, Davis 1938, Rutstein *et al.* 1941, Kojis 1942). For our immediate purpose we need refer only to the following points.

The reaction must be distinguished from the disturbances of the thermo-regulatory mechanism, and the cardio-vascular reactions which are commoner sequels to the injection of serum. It occurs after primary as well as after subsequent injections. It is in no way related to the antibody content of the injected serum, but is a response to the foreign protein as such, which is usually horse serum. The reaction may occur within 2 hours of the injection of the serum, and up to 24 days after, though an incubation period of 8 to 12 days is commonest. Among the "immediate" reactors, there is often evidence of previous treatment with horse serum. Younger people are more prone than older, though the proneness may reflect the fact that younger persons are more liable to have had previous antiserum treatment, as compared with persons whose youth was passed in an age of less extensive serum therapy. The illness itself is characterized by rashes, especially of an urticarial type and often commencing at the site of the inoculation, by fever, joint pains, slight cedema without albuminuria (or with a mere trace of albumin in the urine), and by a varying degree of glandular swelling usually confined to the regional glands that receive the lymphatics from the site of inoculation. The illness is variable in duration, protean in its symptoms, and seldom of a serious nature, though it may be associated with severe discomfort. It is probably never fatal. The nervous system is affected, though rarely. The commonest lesions occur in the 5th and 6th cervical nerves. Neuritis is followed by flaccid paralysis of the muscles, with a slow and, in the greater proportion of cases, a complete recovery. Both motor and sensory nerves are affected. The neuritis may affect one or many nerves, and in some cases the lesions are more central and may even be cerebral. The neuritis is usually ascribed to an urticarial cedema of the nervous tissue (see Davis 1951). It should be noted that these paralyzes may follow the injection of other antigenic substances—vaccines and preparations of toxoid (see Kennedy 1929, Allen 1931, Young 1932, Doyle 1933, Ayer 1935, Wilkinson 1937, Bennett 1939, Hughes 1944).

In a tiny minority of cases an entirely different reaction follows the injection of serum, the patient dying within a few minutes with symptoms resembling those of acute anaphylactic shock. Park (1908) records two such cases among 50,000 persons who received serum injections in New York (see also Park 1921, 1928, Vance and Strassmann 1942).

Acute anaphylactic shock can also follow the injection of antigens other than serum proteins. There are, for example, a number of cases on record of shock after a second dose of a bacterial toxoid; the peptone used in the preparation of the toxoid appears to have been the responsible agent (see Parish and Oakley 1940, Werne and Garrow 1946).

We may note that processes which change the antibody globulin molecule in antisera from the horse also reduce their capacity to cause serum reactions. Thus, when proteolytically refined antisera (Chapter 7) are employed, the incidence of serum sickness and of sensitization to serum sickness, are reported to be greatly reduced (Pohacker-Fritsch and Siegl 1941, Kojis 1942, see also Coghill *et al.* 1940, Jones and Roberts 1941, Top and Watson 1941).

Experimental Serum Sickness and Related Phenomena.—The detailed study of the syndrome that follows the intravenous injection of a single large dose of a heterologous serum into rabbits was initiated by Rich and Gregory (1943), who

may, and often does, vary from one epidemic to another, variation in virulence is not an important factor in the single epidemic wave. Over long periods of time, however, alterations in virulence may contribute to the changes in morbidity and mortality observed in some of the infectious diseases.

The Host Population. In contrast to the relative stability of the bacterial population, the human population is highly variable in its resistance to infection, and the variation, attributable to both intrinsic and extrinsic factors, is not infrequently of such magnitude that its consequences are of considerable practical importance.

Since a population, human or infrahuman, is composed of individual organisms, it follows that its character is determined by the nature of these individuals and their relations to one another, and its reaction to an external influence is expressed in terms of the aggregate of the reactions of its members. The response of a human population to an infectious disease is, of necessity, measured in terms that are composites of the responses of the individual members of the population—in short, by some method of counting. Such counting is, of course, the basis of statistics, and the statistical method, with its ramifications and refinements, is a powerful tool which makes possible the study of the response of human populations to disease—a response which is measured in terms of rates, ratios, life tables and similar numerical devices.

Of the intrinsic factors which determine the response of a human population to an infectious disease, one of the most important is age distribution. The quantitative predominance of the lower age groups characterizing an immature population declines with population growth while the higher age groups correspondingly increase. As a consequence, the diseases of childhood and early adult life, such as diphtheria, tuberculosis and the like, are relatively prevalent in an immature population but become progressively less so with the passage of time, while the diseases of old age increase in incidence with the maturing of the population.⁹ The frequency of an infection is expressed as a rate, either as the number of persons infected in a given unit of time or *incidence*, or as the total number of persons infected at any one time or *prevalence*. Correction of morbidity and mortality rates is a practical necessity and is made either by the use of *specific rates*, i.e., the proportion of cases or deaths within a specified age group, or by the use of *standardized rates* which are not the observed rates but rather what the observed rates would be if the age distribution of the population were that of a standard or reference population.¹⁰

The sex distribution of a population and its racial composition are of somewhat lesser practical significance, although in some communities in the

⁹For a discussion of these problems see Perrott and Holland. *Milbank Mem. Fund Quart.*, 1940, 18 359.

and the populations of states or other portions of the country compared or adjusted to it.

certain persons develop a characteristic symptom-complex as the result of natural contact with some particular substance or range of substances. The substances that may evoke this response are of the most varied nature: in hay fever, pollens of various species; in asthma, pollens, horse dander or the cutaneous débris from other animals; in the food idiosyncrasies, a wide range of different materials; in the drug idiosyncrasies, a whole series of chemical compounds which have no common determinant structure.

One of the most striking features of the idiosyncrasies as a group is that—as in the anaphylactic reaction—the symptom-complex bears no relation to the particular type of substance by which it is evoked. In spite of the varied nature of the exciting materials concerned, the syndrome induced has an essential uniformity; though there are minor differences which seem to depend in the main on the particular route by which the reacting substance gains access to the tissues. It is particularly striking that, in the case of many drugs, the response exhibited by the hypersensitive subject—using hypersensitive in this particular sense—bears no relation to the pharmacological action of the drug in question. From among the drug idiosyncrasies, we must exclude those which are anaphylactoid, due in some cases at least to the capacity of the drug to release histamine from the tissues (see Schachter 1952).

The reactions induced may be either local or general. Among the former are the local reaction to the intracutaneous injection of horse serum or to the application to the skin of such a drug as iodoform, the coryza of hay fever and perhaps the associated asthma, and the acute vomiting and gastro-intestinal disturbance that follow the ingestion of a particular foodstuff. Among the latter are the generalized urticarial eruptions or oedema that may follow the administration by any route of a substance to which a person is hypersensitive, the occasional pyrexia, and the asthmatic symptoms that sometimes follow the entry of the exciting substance by a route other than the respiratory tract. There is also a group of delayed reactions in the skin, exemplified by the "contact dermatitis" following exposure to poison ivy and similar natural products, and to certain chemicals. It is of interest in this connection to note that with the displacement of serum therapy of bacterial infections by chemotherapy, serum sickness has largely been replaced by allergic reactions to the sulphonamides and the antibiotics (see, e.g., Florey 1952).

There are obvious points of similarity between this group of reactions on the one hand and anaphylactic hypersensitiveness on the other, but there are also obvious divergencies.

Among the points of similarity we may include the following (see Coca 1920a).

In both cases the reaction is specific. Although the naturally hypersensitive person may react to several different substances the range is usually limited, and may be confined to a single chemical substance. In both cases the type of response is essentially the same for a single animal species irrespective of the nature of the exciting substance. To this we may add refractoriness of the animal or tissues in which a reaction has been elicited to the same allergen, and in some cases, specific desensitization. We should note also that histamine release has been demonstrated from the blood and skin (Katz 1942), and bronchial tissues (Schild *et al* 1951) of allergic human subjects, on contact with antigen.

Among the features that have been held to distinguish the two conditions are the following. (1) The exciting agent in anaphylaxis is always an antigenic sub-

United States the high mortality rate of the Negro has necessitated the use of race-specific mortality rates.¹¹

The extrinsic factors which alter the resistance of a host population to the spread of disease may exert their effects in either or both of two ways: first, by influencing the resistance of part or all of the individuals comprising the population, and, second, by influencing the relationships between individuals. Perhaps the most important factor in the first category is active individual immunity. If a sufficient portion of a population is immune to a disease as a result of artificial inoculation or recovery from an attack, the resistance of the entire group to epidemics of that disease is of a high order, a phenomenon

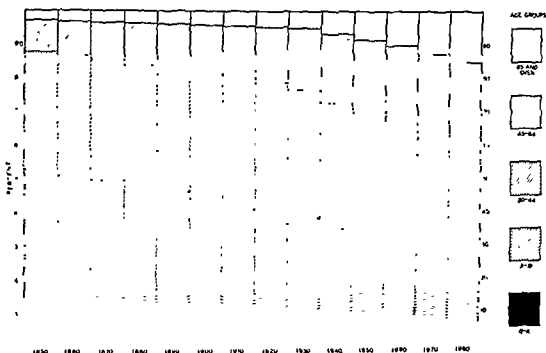


Fig. 31. Changing age distribution in the population of the United States. Estimates 1940 to 1980 by Thompson and Whelpton.

which has been termed *herd immunity*. Other factors may reduce the resistance of the individual, in times of stress or calamity, for example, when relatively large groups are undernourished, fatigued and exposed to inclement weather, epidemic disease may spread with great rapidity.

Equally important to the resistance of a population to epidemic disease are the factors which determine the interrelationships of its members. Crowding in large gatherings or the enforced close association arising from inadequate housing facilities obviously provides opportunity for the dissemination of respiratory and other diseases transmitted directly from man to man, and, as well, certain indirectly transmitted infections, such as louse-borne typhus fever. Similarly, the spread of enteric infections is, to a large extent, dependent upon sanitary facilities and the solution of the twin problems of water supply and sewage disposal. Group practices which support a large rat popu-

¹¹ For a discussion of the methods of population study with respect to disease see Pearl: *Introduction to Medical Biometry and Statistics*, 3rd ed. W. B. Saunders Company, Philadelphia, 1940.

nitrogenous material is removed (Grove and Coca 1925). In these respects atopens do not differ essentially from antigens.

We have seen that many haptens, when injected intravenously into actively or passively sensitized guinea-pigs, will induce acute anaphylactic shock, and we might expect a similar phenomenon in natural hypersensitivity.

There remain instances of drug idiosyncrasies that are not so readily explicable along these lines, since the chemical structure of the exciting agent is not of the relatively complex kind which characterizes those haptens that are capable of inducing acute shock in a sensitized animal.

Numerous observations have, however, indicated a possible mechanism by which such simple compounds might come to function as complete or partial antigens. It has been found that animals may be sensitized by the injection of atoxyl and serum, or even of arsenical compounds alone (see Klopstock and Selter 1929, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931); and it seems probable (Landsteiner and Levine 1930) that in such cases a preliminary union occurs between the simple arsenical compounds and some serum or tissue protein, with the formation of an effective antigenic complex. In later studies Landsteiner and Jacobs (1936) sensitized guinea-pigs with neoarsphenamine, and induced shock by the intravenous injection of guinea-pig serum mixed with arsphenamine, or arsphenamine alone. Jacobs (1932) succeeded in sensitizing guinea-pigs to iodinated sera by injecting them with guinea-pig serum mixed with Lugol's iodine solution, and suggested that a similar mechanism might be involved in the development of iodine sensitivity in man. Horsfall (1934) injected formolized rabbit serum into rabbits, and found that the animals developed skin sensitivity to uncombined formaldehyde. Landsteiner, as part of his investigations of experimental contact dermatitis, induced both skin sensitivity and anaphylactic sensitization by the intracutaneous injection of aromatic substances like picryl chloride and dinitrochlorobenzene (Landsteiner and Jacobs 1936, Landsteiner and Chase 1937) and non-aromatic substances like diazomethane and mustard oil (Landsteiner and di Somma 1938). These substances are all highly reactive and capable of forming conjugates with tissue proteins, and are also known to induce dermatitis or other forms of hypersensitivity in man (see, for example, Wedroff 1932; see also Landsteiner and Chase 1939, 1940b). From a study of the sensitizing action of tetryl (trinitrophenylmethylnitramine) in the skin of guinea-pigs, Gell (1944) concluded that this substance also acted by forming a picryl protein in certain tissues; though there was evidence that not all compounds of tetryl and guinea-pig protein would stimulate sensitivity, and that sensitivity was not necessarily associated with the presence of circulating antibodies to picryl protein. Gell and his colleagues called substances of this kind pro-antigens (see p. 302), whose antigenic potency among other things depended on their power to combine with tissue proteins. The substance 2,4-dinitrophenol is a pro-antigen, and in a study of eight homologous dinitrophenyl compounds, Eisen, Orris and Belman (1952) found that four, which combined irreversibly with skin proteins both *in vivo* and *in vitro*, were strong skin-sensitizing agents, and four which did not had no pro-antigenic activity. It may also be noted that though *p*-phenylenediamine induces skin sensitivity in guinea-pigs, the corresponding ortho- and meta-compounds do not sensitize; and substitution of $-SO_2NH_2$, $-CH_3$ or $-OH$ groups for the $-NH_2$ in the *para*-compound destroyed its sensitizing action (Nitti *et al.* 1937).

Mode of Sensitization in Man; Active and Passive.—The second differential criterion between hypersensitiveness and anaphylaxis is the mode of sensitization. There is no question that human hypersensitiveness occurs naturally and that hypersensitive reactions rapidly develop in persons with no *known* previous contact with the antigen eliciting the reaction. Nevertheless, there is good evidence that this condition frequently arises as the result of active sensitization.

lation make bubonic plague a potential menace, and the presence of large numbers of mosquitoes of the appropriate species allows the wide dissemination of malaria and yellow fever. These and other factors, political, sociological or economic in nature, obviously exert no small influence on the resistance of a population to the spread of disease.¹²

The Interaction of Host and Parasite Populations. It will be clear from the foregoing discussion that the interaction of the host and parasite populations is a highly complex phenomenon. Even assuming that the parasite population, when the parasite is a pathogenic bacterium, remains relatively constant in its ability to produce disease, the resistance of the host population is in a constant state of flux and the equilibrium between the two is rarely a "steady state." As has been indicated, the relation between host and parasite populations is a part of the general problem of interspecies competition, and has been studied at length, particularly by the economic entomologist¹³ and the experimental zoologist.¹⁴ The infectious diseases of man constitute a series of special cases of the host-parasite relationship, differing from one another with respect to mode of transmission, incubation period, period of infectivity, immunity, case fatality, etc. The studies on infectious disease have taken two forms. one, the theoretical analysis of epidemic spread; and the other, the experimental investigation of controlled epidemics among populations of laboratory animals, i.e., experimental epidemiology.

Theoretical Analysis. The theoretical treatment of the dissemination of infectious disease in a susceptible population is exceedingly difficult because of the tremendous number of variables which are involved. If, however, certain simplifying assumptions be made, analysis in terms of the mathematics of probability yields information of considerable significance, and a certain insight into the evolution of the single epidemic wave may be gained.

The evolution of the single epidemic wave is best considered here by the arithmetic method of Frost,¹⁵ which involves finite differences. Let C = the number of cases, S = the number of susceptibles and N = the contacts per day, then r , the contact rate per day, is given by

$$N = rCS \text{ or } r = \frac{N}{CS}$$

It is assumed that each case is infectious, that one contact suffices to produce the disease in a susceptible individual, that multiple contacts are disregarded, and that the unit of time (the day) is small enough so that C and S do not change materially during that period. Then the number of contacts per unit time t (the day) is

$$Nt = rCSt$$

The probability of contact is, therefore,

$$p = \frac{Nt}{S} = rCt$$

¹² Sigerist. *Civilization and Disease*. Cornell University Press, Ithaca, 1943.

¹³ See the reviews by Nicholson. *Jour. Animal Ecol.*, 1933, 2:132. and by Thompson. *Parasitology*, 1939, 31:299.

¹⁴ Gause: *The Struggle for Existence*. Williams & Wilkins Company, Baltimore, 1934.

¹⁵ Frost. Cutter Lecture, 1928, unpublished. Method outlined by Zinsser and Wilson. *Jour. Prev. Med.*, 1932, 6:497

The detailed study of this passive atopic sensitization was taken up by Coca and his colleagues and by other investigators. Coca and Grove (1925) gave the name *atopic reagins* to the antibodies present in the serum of naturally hypersensitive persons, by which the specific hypersensitiveness can be passively transferred to the skin of non-sensitive persons. These reagins were demonstrable in the serum of all those subjects of hay fever or of asthma in whom the reaction to the intradermal injection of the specific atopen was positive. Not all normal skins were receptive, about 11 per cent. failing to react at all, and some 5 per cent. giving slight or doubtful reactions. The remaining 84 per cent. were fully receptive, and the local passive sensitization lasted for at least 4 weeks once it had been established. The reagin could be neutralized by the specific atopen in the test-tube, but gave no precipitin reaction *in vitro*, nor would it induce passive anaphylaxis in the guinea-pig. In a control series of experiments they noted that the normal human skin could not be passively sensitized to egg white or to ragweed-pollen with rabbit antisera, although these contained specific precipitins. Similar phenomena have, however, been noted in the case of passive anaphylaxis; neither horse antisera nor chicken antisera are effective in the passive anaphylactic sensitization of the guinea-pig, though antisera prepared in the rabbit are highly efficient from this point of view. Levine and Coca (1927) found that there was a proportional relationship between the degree of sensitivity of the skin and the reagin content of the serum in 33 cases of hay fever.

Clarke and Gallagher (1926) gave a very clear-cut demonstration of the latent period in passive atopic sensitization and of the *in vitro* neutralization of reagin by atopen without the production of any tissue reaction. They injected 0.05 ml. of atopen into one area of skin, and 5 minutes later they injected 0.05 ml. of reagin-containing serum into the same site and 0.05 ml. of the same serum into a second site some distance from the first. No reaction occurred after these injections, showing that the union in the tissues of atopen and reagin under conditions that give no time for the preliminary fixation of the reagin does not produce the typical response. Next day each site was reinjected with the same quantity of atopen. At the second site, where no atopen had previously been injected, a typical reaction occurred. At the first site no obvious response was elicited, showing that the reagin had been neutralized by the atopen that was present when the sensitizing injection was given.

Reagins and Antibodies.—As outlined above, the reagin differs from antibody in its inability to precipitate specifically with the atopen *in vitro*. Conversely, there is evidence that sera rich in precipitins are not necessarily effective skin-sensitizers.

Otto and Adelsberger (1931) observed that a typical reagin, from persons sensitive to horse dander, was very effective in sensitizing human skin, less so guinea-pig skin, and unable to sensitize guinea-pigs to anaphylactic shock; whereas the sera of persons suffering from serum sickness, or of man, rabbits and guinea-pigs that had produced antibody in response to injections of horse serum, were only moderately effective in sensitizing human skin but quite effective in sensitizing guinea-pigs, both locally in the skin, and to anaphylactic shock. On the other hand, Caulfeild, Brown and Waters (1937) were able to induce the same specific sensitization to extract of ragweed pollen in the skin of a normal person by serum from a hypersensitive person, and by serum from a guinea-pig immunized with ragweed extract; and Straus (1937) found that *rhesus* monkeys were readily sensitized by the injection of human sera containing reagins.

The experimental work with reagins is largely carried out, for obvious reasons, in skin. It should be noted, nevertheless, that passive sensitivity is conferred by the reaguic serum on many other tissues and organs as well (see Walzer 1941).

Skin-sensitizing antibody can also be induced in the rabbit (Cooke and Spain 1929). One pre-requisite for the production in these animals of sensitizing antibody

and the probability of avoiding contact is

$$q = 1 - p = 1 - rCt$$

Since there are $1/t$ units of time in the entire period, the chance of avoiding contact over this period is

$$Q = (1 - rCt)^{\frac{1}{t}} = e^{-rC}$$

Therefore, the chance of at least one contact is given by

$$P = 1 - e^{-rC}$$

and the number of new cases infected during the day is

$$PS = (1 - e^{-rC}) S$$

Assuming the incubation period to be one day—i.e., the contact of one day is the case of the next—a theoretical epidemic wave may be built up by a series of substitutions in the last equation. Starting with 10,000 susceptibles, one case, and a contact rate, r , of .0002, the first day,

$$(1 - e^{-.0002}) 10,000 = 2 \text{ (new cases).}$$

the second day,

$$(1 - e^{-.0006}) 9998 = 6$$

the third day,

$$(1 - e^{-.0018}) 9992 = 18$$

and so on through the complete epidemic wave. Various modifications such as limiting the period of infectivity through the introduction of case fatality and the development of immunity, extending the incubation period, and the like may, of course, be made. This kind of treatment is, of course, an application of the law of mass action. The first relationship, $N = rCS$, becomes $rCS = dC/dt$ and may be manipulated in a variety of ways. Wilson¹⁶ has developed this approach at some length.

Theoretical epidemics built up in this way show a remarkable similarity to observed epidemics of disease, and, although the factors entering into the determination of the value of r are highly complex, it is evident that the probability of chance contact is a factor of primary importance in the evolution of the epidemic wave. In a population consisting largely or entirely of susceptibles, this probability is large and the disease spreads rapidly, but, as the number of susceptibles is reduced by conversion to cases, immunes and fatalities, the probability diminishes and the epidemic subsides.¹⁷ It may be noted that the term "contact" is used to mean "effective contact" and more than one meeting with a case may be required to make up an effective contact. It is often said that an important factor in epidemic spread is dosage, i.e., the number of microorganisms that a susceptible individual encounters within a definite period of time. An adequate "dose" is, clearly, an effective contact.

¹⁶ Wilson and Worcester: *Proc. Nat. Acad. Sci.*, 1944, 30:37, 264, *ibid.*, 1945, 31:24, 142, 203, 327.

¹⁷ For experimental studies on the mathematical theory of epidemics see Kermack and McKendrick *Jour. Hyg.*, 1939, 39:291 *et ante*. Also the summary by McKendrick: *Edinburgh Med. Jour.*, 1940, 47:117.

antibodies could sensitize guinea-pigs to anaphylactic shock with toxoid, but only the precipitin was active in passive Arthus sensitization. The non-precipitating antitoxin co-precipitated with the precipitating antitoxin, thus resembling the "univalent" antibody of Heidelberger (p. 290). Sherman, Menzel and Seebohm (1950) had earlier suggested that rabbit reagins might be "univalent" antibodies (but see Vaughan and Kabat 1953).

As we saw in Chapter 7, there is no reason to suppose that "univalent," or "low-grade," antibody differs essentially from precipitating antibody. Both are associated with the γ and sometimes the β globulin fractions of serum. Reagins, on the other hand, appear to be more commonly associated with α and β globulin fractions (Cooke *et al.* 1951, Campbell *et al.* 1954). They differ strikingly from precipitating antibodies in their affinity for skin tissue, and in heat lability. Heat lability, however, is not an invariable property; rabbit reagins are heat-stable (Sherman *et al.* 1948). Heat lability is tested in whole serum, and it is possible that heating does not directly destroy human reagin, but facilitates the formation of a complex with other serum proteins (cf. p. 252). Antitoxic reagin, indeed, is converted by heat into a specific blocking antibody (Kuhns and Pappenheimer 1952); and an analogous complex formation with serum albumin occurs when precipitating antitoxin is heated, rendering the antibody non-precipitating without affecting its antitoxic potency (Kuhns 1953).

We may, then, regard reagin as one of the general family of antibodies deficient in precipitating power, with physicochemical peculiarities that determine its readiness to adsorb to animal tissues, and, in certain species of animal, to form non-sensitizing complexes with other serum proteins under the influence of heat.

It must be emphasized that skin sensitivity can be induced by precipitating antibodies, and anaphylactic hypersensitivity by reagins. Moreover, the distinction on other grounds is by no means clear cut. We have noted that rabbit reagins are not heat-labile; and Chase (1947) found that hypersensitivity in guinea-pigs induced by simple but reactive chemicals, like picryl chloride, was transferable to normal guinea-pigs by the heat-stable anaphylactic type of antibody which nevertheless, like a reagin, sensitized the skin for periods up to 3 weeks.

We conclude therefore, that there is no case for the term atopen, as a name either of a class of substances distinct from antigens as a whole; or, since both reagins and precipitins may be induced by one and the same antigen, of a special class of antigens. There is perhaps a case for retaining the term reagin to denote non-precipitating antibodies inducing a relatively long-lasting hypersensitivity of the immediate type in skin and other tissues; but not as a name for a class of substances distinct from antibodies in general.

Natural blocking antibodies appear to be more closely related to the full precipitins, as are the blocking Rh antibodies to haemagglutinins (p. 245); they are probably distinct from the blocking antibodies made by heating antisera.

Passive Sensitization by Cells.—The immediate type of reactivity, as we have seen, is often transferable passively in the serum of the hypersensitive animal. Sensitizing chemicals, like the substituted chloro- and nitro-benzenes we discussed on p. 1320, may give rise to antibodies that induce in the normal animal an anaphylactic immediate-type skin sensitivity, which can be elicited by the chemical conjugated *in vitro* with a protein (see Chase 1947); but usually they induce in the actively sensitized animal a delayed type of sensitivity appearing in 5–20 days that is not transferable in the serum.

That sensitization of this kind is dependent on a systemic reaction in the animal was clearly demonstrated by Landsteiner and Chase (1940b), who failed to induce

The nature of herd immunity will become clear at this point, for the higher the proportion of immunes in a population the smaller the probability of effective contact between case and susceptible, *i.e.*, many of the contacts will be with immunes, and the population exhibits a group resistance to epidemic disease which may be of such a high order that an epidemic is no longer possible and the disease smolders in an endemic form as a result of the importation of new cases or the persistence of infection in healthy carriers whose contacts will give rise to an occasional case. A susceptible member of such an immune population, then, enjoys an immunity that is not of his own making but arises as a result of his membership in the group. A measure of this protection is given as Q in the above equations.

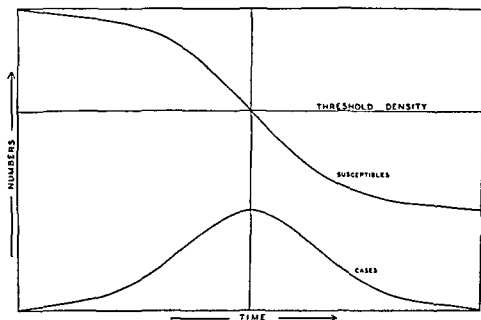


Fig 32. Diagrammatic representation of the course of an epidemic wave in terms of numbers of cases (lower curve) and numbers of susceptibles (upper curve). Note the coincidence of the peak of the epidemic wave with the threshold density of susceptibles (After McKendrick.)

Clearly there will be a critical proportion of susceptibles and immunes in the population, with a greater number of susceptibles epidemic disease can develop and with a lesser number it cannot. This critical point has been termed *threshold density* and is that which prevails at the peak of the epidemic wave. The total number of cases in the epidemic will, then, be twice the number of susceptibles in excess of this threshold density present at the beginning, and following subsidence of the wave the population is left with a greater or lesser degree of herd immunity.

Such precise relationships are only approximated in nature. In practice the threshold density fluctuates and is a function in part of dosage. When the prevalence of a disease is high a given individual will be subjected to a greater number of bacteria per unit time; some, previously immune to smaller doses, will become susceptibles under such circumstances and the

The Genetic Factor in Anaphylaxis and Hypersensitiveness.—The fourth character that has been held to distinguish human hypersensitiveness from anaphylaxis is concerned with the part played by inheritance in the two conditions. There is evidence (Cooke and Veer 1916, Coca 1920a, Spain and Cooke 1924, Clarke, Donnally and Coca 1928) that human hypersensitiveness tends to run in families. As regards the anaphylactic state, which among the allergies has been the most clearly established as due to an antigen-antibody reaction, we should expect that heritable variations in immunizability would be reflected not only in antibody formation but also in sensitization; and, as we saw in Chapter 42, there is evidence that responsiveness to antigens is heritable. But although in passive anaphylaxis the sensitivity is related to the amount of antibody employed, there is no evidence to indicate how far sensitivity is associated with the degree of *active* immunization, or whether such an association is hereditarily determined.

The incidence of natural human hypersensitivity is, of course, no direct measure of the proportion of the human race that has an hereditary disposition to sensitization. Among other things the opportunity for experience of the antigen, the intensity and duration of that experience, the nature and the route of entry of the antigen both as a sensitizer and an inducer of the sensitive reaction, will affect the issue. But when we can subject a sample of the population to a fixed sensitizing dose, we find a significant variation in response. In experimental sensitization of man with an extract of a *Primula* sp., for example, Bloch and Steiner-Wourlich (1926) observed that, whereas some persons were sensitized with one application, others required several applications.

With regard to the inheritance of human hypersensitiveness we have already noted (see Cooke and Veer 1916, Spain and Cooke 1924) that the genetic factor appears to be concerned with a tendency to develop reaginic hypersensitiveness in general, rather than a sensitiveness to any one antigen in particular. It appears to be unusual to find that parents and offspring, or even the same substance, or one may be sensitive to cat hairs, and so on. It is the type of hypersensitive reaction in general appears to be in some degree inherited; the tendency to develop asthmatic symptoms, for instance, being particularly frequent in certain families.

Among the 131 young adults they tested, Kuhns and Pappenheimer (1952) found 40 that were hypersensitive to diphtheria toxoid; of these, 35 (87·5 per cent.) had a personal or a family history of allergy. Put in another way, the incidence of hypersensitivity was 60 per cent. in 59 subjects with a positive history and only 7 per cent. in those with a negative history.

Landsteiner and Chase (1940a) report that guinea-pigs can by breeding be separated into strains of high and low susceptibility to sensitization by dinitrochlorobenzene, or to the atropens of poison ivy, thus affording one indication of a similarity between man and experimental animals in a heritable tendency to develop hypersensitivity.

There are insufficient grounds for postulating an essential difference between anaphylaxis and hypersensitiveness on the premises of our present knowledge of the influence of genetic factors in the two conditions.

Desensitization in Hypersensitiveness and Idiosyncrasy.

Specific Desensitization.—With regard to the fifth distinctive character, some observers, and particularly Coca (1920b), lay great stress on the difficulty of desensitization in the sensitive human subject as compared with its relative ease and

"effective concentration," so to speak, of immunes declines with consequent effect on the herd immunity to epidemic disease. This effect of dosage on the host population has been termed *infection pressure* and is a matter of considerable practical importance. Successive epidemic waves may, then, occur as a consequence of increased infection pressure as well as through the accumulation of new susceptibles (see below). Recurrent waves of a disease that is a clinical but not an etiological entity are, of course, another matter.

The question of the relationship between an infectious disease and a susceptible population over a long period of time is somewhat more complex. Mathematical treatment such as the Ross malaria equations and Martini's equations for immunizing diseases¹⁸ is too far removed from reality to have much practical significance, principally because the mathematical approach requires that the parameters remain constant over long periods of time. This condition is probably rarely if ever satisfied; it is well known, for example, that within the last century both diphtheria and scarlet fever, previously of low prevalence, took on a malignant character and higher prevalence for some decades and then within the last forty years have gradually declined in both prevalence and severity. It is of some interest, however, that the differential equations of Martini, for example, show one stable equilibrium at the origin and another at a positive value which is approached by a series of oscillations above and below the final state of equilibrium so that a series of epidemic waves appears. These solutions suggest that (a) under certain circumstances a disease may tend to die out and (b) under other circumstances a disease will reach an equilibrium after a series of epidemics of decreasing severity.¹⁹ Such periodicity in the incidence of many infectious diseases is well known, one of the best examples is that of measles which recurs in epidemic form at approximately two-year intervals. So far as is known, however, no infectious disease occurring under natural conditions shows a damped periodicity approaching invariability though it has been found to occur in experimental epidemics in which the immigration rate of the new susceptibles is very high (*vide infra*).

A disease will, of course, die out if it does not reproduce itself, i.e., if the bacterium is present only in the active case and each case does not, on the average, give rise to a second case. It is not unlikely that some diseases are dying out in this way, it has been suggested,²⁰ for example, that the decline in tuberculosis is due in part to inability of the disease to reproduce itself, a process accelerated by isolation of active cases. The second suggestion is of interest in connection with the epidemic periodicity of certain diseases such as measles, which appears in epidemic form usually at two year intervals. It is obvious, of course, that following an epidemic a new crop of susceptibles appears, and when their numbers reach a sufficiently high level, a new epidemic ensues and so on *ad infinitum*. It is unlikely, however, that these epidemic waves are damped as predicted by theoretical equations, for subtle variation in the complex of factors that are oversimplified as parameters

¹⁸ For a discussion of this general problem see Lotka: *Elements of Physical Biology*. Williams & Wilkins Company, Baltimore. 1925.

¹⁹ See Wilson and Worcester: *Proc. Nat. Acad. Sci.*, 1945, 31:294.

²⁰ Frost: *Amer. Rev. Tuberc.*, 1935, 32:644.

Delayed reactions of the tuberculin type are definitely diminished, even by relatively small doses of the hormone (Long and Favour 1950, Long and Miles 1950, Harris and Harris 1950). There is in rabbits an associated suppression of the characteristic reticulo-endothelial cell reaction (Gell and Hinde 1951; see also Osgood and Favour 1951).

Sensitization of guinea-pigs to tuberculin is also decreased by cortisone, but the capacity of lymphocytes to transfer hypersensitivity is not altered (Cummings and Hudgins 1952). (For other references, see the review of Kass and Finland 1953.) The action of cortisone is obscure. Long and his colleagues (see Long 1954), who described in guinea-pigs a tuberculin desensitization by ascorbic acid, sought to relate it to control of amino-acid metabolism, which in turn affects the oxidation of ascorbic acid and the accumulation of glucose-1-phosphate, an intermediate of carbohydrate metabolism; experimentally the phosphate ester proved to be a powerful desensitizing agent.

Summarizing, it appears that reactions involving a proliferation of mesenchyme cells, whether antibody-forming cells during sensitization, or the inflammatory cells associated with delayed-type reactions, are diminished by the hormone; but that the immediate, purely exudative reactions are little altered. In any event, the reaction of antigen with antibody does not appear to be affected.

ALLERGIC REACTIONS IN BACTERIAL INFECTIONS

The term *allergy* is almost impossible to define precisely. We use it in a loose, but widely accepted sense to cover a group of reactions characterized by a heightened or accelerated response to a particular type of antigen, irrespective of the balance of harm, or benefit, that the altered response confers on the allergic host.

All the phenomena of hypersensitivity could obviously be included under this general label. In the present section we are concerned with those instances in which an allergic state develops in relation to a bacterial infection.

It has already been noted that bacterial haptens may induce acute anaphylactic shock in a sensitized guinea-pig; but before discussing allergic reactions in the wider sense we may refer briefly to anaphylactic sensitization with bacterial antigens.

Active anaphylactic sensitization has been induced with various bacterial antigens and passive sensitization with the corresponding antibodies (Rosenau and Anderson, 1907 Kraus and Doerr 1908, Holobut 1909, Kraus and Admiridzibi 1910, Zinsser and Parker 1917, Tomesik and Kurotchkin 1928, Lancefield 1928, Enders 1929, Avery and Tillet 1929), or by Dale's technique with the isolated uterus (Zinsser and Parker 1917, Zinsser and Mallory 1924, Tomesik and Kurotchkin 1928). The reactions are specific and desensitization can be demonstrated. Sensitization to bacterial antigens cannot, however, be produced with the same ease as can sensitization to such antigens as horse serum or egg white. A single injection of bacteria frequently fails to produce any demonstrable sensitization, and the most successful results have been obtained by giving repeated small injections of antigen, followed by an interval of three weeks or so before the intravenous injection of the test dose, which should be relatively large. A technical difficulty in work of this kind is introduced by the fact that many of the bacterial suspensions employed are themselves toxic when administered in any considerable dose, and the symptoms that develop in normal animals after the injection of massive doses of bacteria or bacterial products may be very similar to anaphylactic shock. The difference in the response of normal and of sensitized animals may, therefore, be relatively difficult to detect.

will wipe out the damping effect. It will probably be many years before predictions regarding the future of infectious diseases attain a status better than that of guesses.

Experimental Epidemiology.²¹ The information that may be derived from observation of naturally occurring disease, i.e., descriptive epidemiology, is limited since the observer has no control over the process and for all practical purposes the experiment is carried out for instead of by him. In experimental epidemics, however, the conditions may be adjusted as desired and it may be anticipated that such experiments will be highly informative.

In recent years the study of epidemics of infectious disease developed in populations of laboratory animals under controlled conditions has been carried out by Topley, Greenwood, Wilson and others in England and by Webster and his colleagues in the United States.²² These experimental epidemiological studies have been confined, for the most part, to the study of the dissemination of *Salmonella typhi-murium* (aertrycke), *Salmonella enteritidis*, *Pasteurella muriseptica* and the virus of ectromelia (a foot-pad disease of mice) among populations of mice. Mouse typhoid and pasteurellosis are regarded as analogous to human diseases such as typhoid fever in which there is an imperfect immunity and carrier state, and ectromelia as analogous to human diseases in which a solid immunity is developed, such as diphtheria. The extent to which the analogies may be carried, and to which the conclusions reached are applicable to the considerably more complex human population as it exists under natural conditions, is open to some question, nevertheless, these studies, as yet in their infancy, have yielded valuable information.

The experiments which have been carried out were of two general types. The closed epidemic was produced in a population of mice, often about fifty in number, by the introduction of infected animals. The other type of experiment was carried out in an infected mouse population recruited by continuous immigration, i.e., mice were added at regular intervals, the rate varying from one mouse every three days to six mice per day. The results may be summarized briefly:

(1) The epidemic wave initiated in the closed population by the introduction of infected individuals closely resembled those observed in the human population.

(2) The effects of dispersal of the infected population of the closed epidemic into large or small groups at various times during the development of the epidemic were studied. The time at which the population was dispersed was found to be of primary importance; the later dispersal was effected, the less favorable the result, and after the peak of the epidemic wave was reached it continued unchecked even though the population was dispersed to individual mice. This is of particular interest since fleeing from an epidemic has been a popular method of escaping infection, viz. the *Decameron*. In this connection the results of dispersal of children from the industrial centers of England in 1939-1940 may be noted. A sharp reduction of 40 per cent or more in diphtheria morbidity occurred in the evacu-

²¹ See the general discussion by Topley: *Proc. Roy. Soc., Ser. B*, 1942, 130-337.

²² See the review by Greenwood, Hill, Topley and Wilson: *Medical Research Council Special Report Series No. 209*, 1936. Webster: *Medicine*, 1946, 25-77.

a relatively short interval a local chancre does not develop (Truffi 1910); but reinfection with a different strain may induce a typical local lesion (Kolle and Schlossberger 1926). Brown and Pearce (1921) infected a number of rabbits and treated some of them with arsphenamine shortly after the development of the primary chancre. Five days later these rabbits were injected with the same strain of *Trep. pallidum*. Most of the treated animals developed chancres. The untreated animals did not.

Kolle (1922, 1924) treated rabbits with neosalvarsan at various stages of infection. He found that rabbits treated within about 6 weeks after the initial infection usually responded to a subsequent injection by developing a new chancre. When treatment was delayed beyond this point, reinfection seldom gave rise to a chancre. When treatment was delayed for about 3 months a new chancre could very rarely be produced. Rabbits that were treated and reinfected at long intervals after the primary infection (75-250 days) were not, however, completely resistant to reinfection (see Kolle and Prigge 1927, 1929). Tissue extracts from treated rabbits that had not been reinfected were non-infective for normal rabbits, but similar extracts from treated and reinfected rabbits gave rise to characteristic lesions, showing that the spirochaetes had gained access to the tissues and were presumably multiplying in them, at least to a limited extent. On these grounds Kolle and his colleagues maintain that the immunity to reinfection displayed by rabbits that have been treated in this way is only a "chancre immunity." The exact significance of this term is a little dubious. We have noted many examples of a partial antibacterial immunity in which bacteria may persist for long periods in the tissues without giving rise to any of the lesions that they produce in non-immunized animals. Moreover, the evidence suggests that a more complete immunity against *Trep. pallidum* may be induced under optimal conditions. Uhlenhuth and Grossmann (1928) treated syphilitic rabbits with neosalvarsan 107 to 506 days after the primary infection. Attempted reinfection 3 to 10 months after treatment gave rise to no obvious lesions; and in only 4 of 11 rabbits could spirochaetes be demonstrated in the tissues.

The results of experiments where the cures were obtained with the more efficacious penicillin even more clearly indicate an immunity persisting after the destruction of the spirochaetes (see Chapter 81; and, for a comparable state of affairs in a bacterial disease, brucellosis, see Pollack *et al.* 1952).

The point at issue is not whether immunity to reinfection, and "symptom-immunity," exist in animals with demonstrable infection; that is clearly established: but whether immunity is necessarily dependent on the infected state. As Staritsky (1948b) points out, in proofs based on demonstrable susceptibility of apparently cured animals and the refractoriness to reinfection of those thought to be infected, there is sometimes a circular argument wherein animals proving susceptible to reinoculation are assumed to have been cured of the first infection, and those proving refractory are assumed still to be infective.

(This discussion of infection immunity, it should be noted, is confined to bacterial diseases. In Chapter 55 we discuss a well-established type of infection immunity which so far has proved characteristic of virus diseases—where the infection of susceptible cells by one virus may prevent their infection by a second virus; here the immunity appears to result from the occupation by the first virus of certain key metabolic processes of the host cell upon which both viruses depend for their proliferation.)

In summary, we may say that immunity to reinfection appears to be established within a short time after the appearance of the initial lesion. At this stage the maintenance of immunity is associated with the maintenance of infection; complete chemotherapeutic cure may be followed by a return of susceptibility. But after a longer period of infection a partial immunity is established that may persist after complete chemotherapeutic cure, and, after still longer periods of infection, a complete immunity may be established that is independent of the persistence of infection.

ated towns together with an increase of 60 to 70 per cent among local children in reception centers, the latter returning to normal within six months. Similarly, the biennial periodicity of measles was broken by this dispersal.²³

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(4) In the mouse population recruited by continuous immigration a series of epidemic waves developed, the frequency of which was directly related to the rate of immigration. Thus, with a low rate, such as one mouse every three days, epidemic waves were separated by periods of remission in which no deaths occurred. As the immigration rate was increased, the waves occurred with greater frequency and with the addition of six mice per day there were no periods of complete remission, the epidemic waves taking the form of periods of increased mortality.

(5) With high immigration rates, the peaks and troughs in the daily death rate became less and less pronounced after a year or more, both the rate and total population tending to become invariant. Thus it was possible to approximate experimentally the damped periodicity and eventual equilibrium predicted on theoretical mathematical grounds (see above), and the experimental host population could be brought into approximate equilibrium with the parasite population.

(6) An inadvertent experiment with a population recruited by a high rate of immigration proved to be of considerable interest. The mouse population had reached the equilibrium indicated above by summer when there was a heat wave of unusual intensity in London. There were many non-specific deaths (mice are highly susceptible to heat), followed by violently fluctuating specific mortality. In the course of time an equilibrium was again reached but at a considerably higher death rate and decreased numbers than formerly. The parasite was shown to be unaltered in virulence and the immigrants had not, of course, had the heat experience. Hence the host-parasite relationship *per se* was altered by an environmental "catastrophe." The relationship of these observations to the long term relation between the human population and infectious disease is of interest. If it be assumed that the day of the mouse is equivalent to thirty days in the life of man, over the four years taken by this experiment the following were observed: (a) a period of stability with low regular death rates and growing population (ca. 600 days) equivalent to a human experience of slightly less than fifty years—this, it may be noted, is longer than the twenty-five year period ending about 1830 during which the death rate from scarlet fever was relatively low; (b) a period equivalent to fifteen years of severe and repeated epidemic waves; and (c) a period equivalent to some thirty years of relatively high mortality in a more or less stable state of equilibrium in a population reduced in numbers—it may be noted that the post-1830 epoch of virulent scarlet fever lasted more than forty years.

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²³ Stocks: Jour. Roy. Statistical Soc., 1941, 104:311, *ibid.*, 1942, 105:259.

sensitized cells, protects the antibody that is fixed to the cells, except when an excess of antigen is injected.

The difference between the anaphylactic and immune state is quantitative rather than qualitative, depending upon the balance between circulating and fixed antibody.

(6) Many of the most dramatic features of the syndrome of anaphylactic shock are the result not of the antigen-antibody reaction itself but of the liberation, among other substances, of histamine by the injured cells, and the consequent response of the histamine-sensitive cells throughout the body. The histamine acts chiefly upon the vascular endothelium, promoting exudation of blood fluids; and upon smooth muscle, making it contract. Acute local reactions may be elicited in the tissues of anaphylactically sensitive animals. Isolated preparations of smooth muscle like the intestine react to antigen by contraction; and local regions of the skin of the intact animal react by increased permeability of the blood vessels and acute exudation of blood fluids.

(7) The sensitized animal may be desensitized by the injection of the specific antigen in amounts too small to induce acute shock or by a route that ensures slow absorption so that there is no rapid transport to the circulating blood.

(8) A variety of substances, when injected intravenously, give rise to a syndrome resembling, in many but not in all particulars, that of anaphylactic shock. The resemblance of these anaphylactoid reactions is not due to any similarity in the underlying mechanism, but to the fact that, in each case, cellular injury is followed by the liberation of histamine and other substances, and this by the characteristic syndromes of histamine shock.

(9) In man, the "immediate" type of hypersensitivity, corresponding to acute anaphylactic hypersensitivity, is induced by certain drugs; by antigens such as pollen, horse serum and horse dander; and by bacterial proteins. It is found characteristically in hay fever, in some cases of asthma, and in urticaria. The antibody concerned is often non-precipitating, with a high tissue affinity ("reagin"). It is detected by passive transfer to normal skin, which then acquires a local specific hypersensitivity.

Sensitizing chemicals like picryl chloride, and plant substances from *Primula* spp. and poison ivy, become antigenic by forming complexes with the body proteins, and so induce hypersensitivity. They induce a delayed reaction on application to the skin. Combination with the skin proteins may in part explain the delay, because these substances elicit immediate reactions when conjugated *in vitro* with protein. The antibodies concerned may be either precipitating or non-precipitating. In some cases sensitivity can be passively transferred by the serum, in others, it is transferable only by lymphocytes.

In these natural allergies, temporary desensitization by treatment with antigen is induced with greater difficulty than in experimentally produced hypersensitivity.

(10) The Arthus reaction, induced by repeated intracutaneous injection of antigen into one site, is characterized by a massive granulocyte reaction and severe damage to blood vessels, and appears to depend on the intravascular combination of antigen with antibody. A similar localization of the antigen-antibody combination is associated with the development of the various vascular lesions found in serum sickness.

(11) Specific hypersensitiveness may also develop in the course of natural or experimental bacterial infection. This type of hypersensitivity is characterized by

of time that they were members of the infected population; the rate rose rapidly to a peak in the early days of cage age and, although it declined slowly with the passage of time, to a greater extent in ectromelia than in mouse typhoid or pasteurellosis, the survivors of one epidemic wave may be the victims of another. The removal at regular intervals of a number of mice equal to the number added at the same intervals markedly altered the trend of these mortality rates; under these circumstances, the initial peak of mortality was lower, but the rapid decline was not apparent and the mortality rate remained at a high level.²⁴ Removal was of considerable advantage to the individual mouse; in general, the earlier the removal the greater the advantage, except during the decline of an epidemic wave, when isolation did not increase the chances for survival of individual animals.

Although the evolution of the single epidemic wave in the experimental studies closely resembles its counterpart in the human population, the inability to control experimental epidemics by immunization is at variance with observations on epidemic disease in human populations. Some human diseases, such as diphtheria and smallpox, can be restrained from assuming epidemic proportions by active immunization of a sufficiently large number of the individuals making up the population. Whether or not this and certain other minor discrepancies prove to be real or illusory with future work, a number of pertinent suggestions have come out of these investigations. The response of the experimental host population in terms of mortality rates and cage life expectation cannot be interpreted as yet in some cases; in others the interpretation is questionable. For example, the English workers feel that some of the data suggest alterations in virulence and "infectivity" of the bacteria, while Webster in the United States interprets these findings as indicative of a graded, inheritable resistance in the host population.

Some of the experiments, however, have yielded clear-cut results that bear directly upon the interaction of host and parasite populations in nature. Perhaps the most important of these is the experimental demonstration of recurring epidemic waves resulting from the continuous addition of new susceptibles to the infected population. In spite of the fact that such a sequence of events is predicted by theoretical epidemiology, as indicated above, the repeated flaring up of a disease of man or domestic animals, thought to be stamped out in the inter-epidemic periods, is most often taken as evidence of the reimportation of infection.²⁵ The experimental demonstration of this

²⁴ Greenwood, Hill, Topley and Wilson: *Jour. Hyg.*, 1939, 39:109.

²⁵ In this connection Major Greenwood (*Epidemics and Crowd-Diseases*. The Macmillan Company, New York, 1935) has made the following delightful suggestion: "... a majority of the officials who in different countries report upon epidemics among farm animals cannot bring themselves to conceive that upon a farm or within a district wherein a scheduled disease once existed, and was afterwards officially declared to have ceased

therefore, to read the Dutch or German explanations of the recrudescence of foot and
bizarre."

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of time that they were members of the infected population; the rate rose rapidly to a peak in the early days of cage age and, although it declined slowly with the passage of time, to a greater extent in ectromelia than in mouse typhoid or pasteurellosis, the survivors of one epidemic wave may be the victims of another. The removal at regular intervals of a number of mice equal to the number added at the same intervals markedly altered the trend of these mortality rates; under these circumstances, the initial peak of mortality was lower, but the rapid decline was not apparent and the mortality rate remained at a high level.²⁴ Removal was of considerable advantage to the individual mouse, in general, the earlier the removal the greater the advantage, except during the decline of an epidemic wave, when isolation did not increase the chances for survival of individual animals.

Although the evolution of the single epidemic wave in the experimental studies closely resembles its counterpart in the human population, the inability to control experimental epidemics by immunization is at variance with observations on epidemic disease in human populations. Some human diseases, such as diphtheria and smallpox, can be restrained from assuming epidemic proportions by active immunization of a sufficiently large number of the individuals making up the population. Whether or not this and certain other minor discrepancies prove to be real or illusory with future work, a number of pertinent suggestions have come out of these investigations. The response of the experimental host population in terms of mortality rates and cage life expectation cannot be interpreted as yet in some cases; in others the interpretation is questionable. For example, the English workers feel that some of the data suggest alterations in virulence and "infectivity" of the bacteria, while Webster in the United States interprets these findings as indicative of a graded, inheritable resistance in the host population.

Some of the experiments, however, have yielded clear-cut results that bear directly upon the interaction of host and parasite populations in nature. Perhaps the most important of these is the experimental demonstration of recurring epidemic waves resulting from the continuous addition of new susceptibles to the infected population. In spite of the fact that such a sequence of events is predicted by theoretical epidemiology, as indicated above, the repeated flaring up of a disease of man or domestic animals, thought to be stamped out in the inter-epidemic periods, is most often taken as evidence of the reimportation of infection.²⁵ The experimental demonstration of this

²⁴ Greenwood, Hill, Topley and Wilson: Jour. Hyg., 1939, 39.109.

²⁵ In this connection Major Greenwood (*Epidemics and Crowd-Diseases*. The Macmillan Company, New York, 1935) has made the following delightful suggestion: "... a majority of the officials who in different countries report upon epidemics among farm animals cannot bring themselves to conceive that upon a farm or within a district wherein a scheduled disease once existed, and was afterwards officially declared to have ceased to exist, it could start into life without a reimportation of *materies morbi*, these happenings must be explained by reimportation. We can always see a joke much more easily when it is against some poor foreigners rather than against ourselves. I advise an Englishman, therefore, to read the Dutch or German explanations of the recrudescence of foot and mouth disease and learn how the virus may be blown by the winds of heaven, or dropped on an island in the excreta of wild geese. A Dutchman might find explanations of the reimportation of foot and mouth disease to be found in English official documents equally bizarre."

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phenomenon and the indicated futility of quarantine in the control of a disease which has become widely disseminated (only when a disease is not present, or present in limited effective foci, can quarantine and isolation procedures be effective) is, clearly, of considerable significance.

Epidemiological Data and Their Interpretation. The purposes of epidemiological study are threefold: first, to indicate the nature of the infective agent, its source and modes of transmission when these are not fully established otherwise, i.e., on an experimental basis; second, to extend this information into a corresponding general theory of the epidemiology of the disease; and third, to determine in detail the local conditions which favor or control the dissemination of the infection in a given area or community.

For these purposes four general types of information, usually simple in character but wide in extent, are required. First, the *area* in which the disease occurs and the regularity of its geographical distributions are highly informative. Thus, the general occurrence of a disease indicates that environmental conditions, including fauna, climate, etc., peculiar only to parts of the area are not essential to its transmission. Similarly, restriction of the disease within geographic limits indicates that special environmental conditions are necessary for its dissemination; these may include crowding, presence of an insect vector, water supplies, proximity to reservoirs of infection, etc. In general, a uniform distribution of the disease is indicative of a simple method of transmission, as in the case of measles, whereas an irregular distribution, such as that of spotted fever, implies a more complex process dependent upon a source of infection or conditions necessary to transmission that are correspondingly irregularly distributed.

Second, the *rate of prevalence* of the disease suggests the source of infection. A high rate, such as that of measles, indicates that the observed cases are the most important, if not the only source of infection. Conversely, sporadic distribution of widely separated cases implies the existence of a concealed reservoir of infection such as the casual or chronic carrier or some of the lower animals.

Third, the *seasonal distribution* is also informative when considered together with other epidemiological features of the disease. Thus, a seasonal occurrence may be consistent with hypothesized insect transmission or it may eliminate an insect vector as the sole transmitting agent. Similarly, the occurrence of water-borne typhoid fever in the late winter and early spring supports the hypothesis of preservation of typhoid bacilli in the cold and their liberation from contaminated watersheds in the first thaws.

Lastly, the *age distribution* of the disease is suggestive as a corollary to other epidemiological characteristics, and often aids in their interpretation. Thus, a disease occurring for the most part in the early years of life, such as measles or diphtheria, often shows differences in age incidence between urban and rural areas which are explained by the more frequent occurrence of immunizing infections, apparent or subclinical, in the more crowded areas. Since similar differentials are apparent in poliomyelitis, the age distribution of this disease, together with other of its epidemiological features, supports the view that this disease is widely prevalent as a subclinical infection.

CHAPTER 52

CERTAIN NON-SPECIFIC MECHANISMS IN GENERAL IMMUNITY

THE protective mechanisms we have discussed in the preceding chapters are specific in their action. Whether established by nature or by art, they increase the resistance of the body against some particular bacterial infection, not against bacterial infections in general. In the present chapter we shall consider certain mechanisms from which this element of specificity is absent.

Many of these non-specific activities cannot be easily related to defence as a whole. Often they relate to little more than the observation of an antibacterial action by body fluids or tissues, or by their derivatives; and even when the antibacterial element in the system under observation has been identified with some precision, we may be unable to estimate its significance, either quantitatively or qualitatively, in natural infection. Sometimes the activity is lacking in certain animals—as for example in the breed of guinea-pigs without complement in the blood we noted in Chapter 42—or it may be associated with a resistant species of host, thus enabling us to estimate its relation to immunity; but such estimates must be made with caution, because the differences we observe may be accidental, or associated with unrecognized changes in other more important elements of the defence system.

Thus uncertainty limits us in this chapter mainly to description. It is an uncertainty that characterizes many investigations of non-specific mechanisms. By contrast, the certainty of the many conclusions we have drawn about specific immunity is a measure of the ease with which we can investigate substances, such as antibodies, that have nicely distinguished specificities.

Blood Factors

β -Lysins, Leukins and Plakins.—Among the earliest records of experimental immunity are some observations on anthrax infection, which established clearly the presence in the serum of certain animal species of bactericidal substances different in kind from the bactericidal complex formed of specific sensitizer and complement. Thus the serum of the rat is actively bactericidal for the anthrax bacillus, and this activity remains after heating the serum at a temperature of 56–60° C. for 30–40 minutes (von Behring 1889, 1892, Malvoz 1902, Pirenne 1904). The name *β -lysins* has since been applied to these relatively thermostable bactericidal serum constituents.

Other early investigations showed that similar thermostable bactericidal substances could be extracted from suspensions of polymorphonuclear leucocytes. Like the *β -lysins*, these *leukins* are restricted in their range of activity, some bacterial species, in particular the spore-bearing aerobes such as *B. anthracis* and

Epidemiological evidence, therefore, consists of a series of inter-related facts from which a conclusion or series of conclusions may be drawn. The first step in epidemiological procedure is necessarily the demonstration of associations between the frequency of occurrence of the disease and some conditions or set of conditions, the second the ascertaining of the relationship of these associations with one another, and third their relations to the general epidemiological theory of the disease. To take a simple example, in milk-borne typhoid fever it may be ascertained that cases of the disease occur predominantly along the route of the milkman, that in families so supplied the cases occur among those who drink milk, that the cases appeared within a limited time to suggest simultaneous infection and that an employee of the dairy is a carrier of typhoid bacilli. Not only are these associations related to one another but they are consistent with the general epidemiological theory of typhoid fever, viz., that transmission is basically a matter of the transfer of infected fecal material (or urine) from a case or carrier to the mouth of a susceptible person.

While this would appear self-evident, there is an opinion of some prevalence that since epidemiological evidence is necessarily purely circumstantial, it cannot be conclusive. This is perhaps due in large part to failure to appreciate the development, often basically statistical and therefore mathematical, of the logical analysis, and the significance of the body of evidence as a whole. The method of analysis and interpretation of epidemiological evidence is identical with that of experimental evidence, the practical difference lying in the fact that, as pointed out earlier, the epidemic is an experiment done for rather than by the observer and cannot be manipulated.

The Control of Infectious Disease. With an understanding, albeit admittedly imperfect, of the factors which determine the means and extent of the dissemination of infectious disease, comes the possibility of control. The variety of epidemiological types of disease and the individual variability within these broad groups make for differences in control measures, and the control of a given disease under given circumstances almost always constitutes a special case. Certain generalizations may, however, be made which will serve to indicate the complex nature of the problems of the control of the spread of infection and provide a point of departure for consideration, in later chapters, of specific diseases.

In general, control measures may be regarded as corollaries of the factors involved in the transmission of infection and, therefore, fall into two groups: one including those which raise the resistance of the individual and, through him, of the population; and the other, those designed to alter the relationships of the individual members of the population to one another in such a way that the opportunities for the dissemination of the infectious agent are reduced. Very often both types of control measures are used, their relative efficiency and practicality varying from one disease to another.

Diseases of lower animals which are transmitted either directly or indirectly to man involve a third population, the animal reservoir of infection. When transmission is from animal to man and occurs only rarely from man to man, three methods of control are possible. First, the disease may be controlled in the animal, second, the relationship between the human and

activity of leucocytes, in both phagocytosis and chemotaxis (Delaunay and Pagès 1946, Delaunay *et al* 1951).

Clearly there exist in the normal sera of certain animal species bactericidal substances which are non-specific in their action, in the sense in which immunological specificity is generally understood—though their range of activity is limited by the varying sensitiveness of different bacterial species—and which are not increased in amount as the result of immunization. Bactericidal substances having very similar properties may be extracted from leucocytes and perhaps from blood platelets.

The relation of these substances to effective immunity is by no means clear. To take the case of anthrax, the relatively resistant rat has β -lysins in its serum; so has the susceptible rabbit, but the relatively resistant dog has none (see Ledingham 1922). The significance of the leukins and plakins is still more difficult to assess. They are not identical with leucocytic enzymes (see Fleischmann 1928), though the participation of extracellular enzymes of this kind in leukin action cannot be excluded. That the antibacterial substances present in or derived from leucocytes play some part in resistance to infection seems highly probable; but we do not know whether their rôle is primary or secondary, nor how it is related to other defence mechanisms.

Tissue Factors

Antibacterial Substances in the Tissues.—Bactericidal substances have been sought for in normal tissues, in attempts to explain their natural immunity, and in infective tissues, in attempts to explain the destruction of bacteria that often follows the local reaction to invasion.

Nutini and Lynch (1946) added extracts of brain and spleen to cultures of toxigenic *Staph. aureus*, and obtained a non-toxigenic variant; they also found that the extracts protected mice to some extent against infection with a toxigenic staphylococcus. Micks, Whitney and Anigstein (1951) isolated from hydrolysed human and cattle red cells a polypeptide which cured streptococcal infection in mice. Bloom and his colleagues (see Bloom and Prigmore 1952) obtained substances, either basic proteins or polypeptides, active in varying degree against *B. anthracis*, other *Bacillus* spp., *Bact. coli* and *Str. pyogenes*, from the thymus, pancreas, thyroid and other mammalian organs. The authors assigned a prominent rôle to the anthracidal factor in immunity to anthrax (see Chapter 76). Another tissue constituent, the basic protein spermine, is bacteriostatic and sometimes bactericidal for a number of bacteria (see Rozansky *et al* 1954). Hirsch and Dubos (1952, Hirsch 1953) isolated it from kidney, and found it to have a powerful bacteriostatic action on the tubercle bacillus *in vitro* when activated by another tissue constituent, spermine oxidase. It was less effective on saprophytic mycobacteria. They also extracted a tuberculostatic basic polypeptide from the thymus, spleen, and pancreas of the calf. It was not present in calf lung or liver, suggesting that its distribution in various organs might be inversely related to the distribution of tubercles in the natural disease (Dubos and Hirsch 1954; see also Bloom *et al* 1953, Fletcher *et al* 1953). Tuberculostatic substances have also been described in the lymph nodes, spleen and liver of tuberculous animals (Soltys 1953). Lewis and Schwartz (1949) briefly reported the *in vitro* killing of *Str. pyogenes* by an extract of rat liver. The factor was not present in other rat organs tested and did not affect three other species of bacterium examined.

Very little can at present be said about the significance or mode of action of these tissue substances in immunity; we may note, nevertheless, that many of them, like the antibiotics derived from bacteria (p. 189), are basic peptides.

animal populations may be altered in such a way that transmission of infection cannot take place; and third, the resistance of the human population may be raised by immunization procedures. The relative efficacy of these methods of control is variable and depends upon the disease. Rabies, for example, can be controlled in the dog population, and, in fact, has been entirely eliminated in England by rigid regulation, but neither mass immunization of the human population nor alteration of the relationship between dog and human populations is practical. In the case of bovine tuberculosis and undulant fever, however, disease may not only be controlled in the animal population, but the relation between human and animal populations is readily altered by interposing the barrier of pasteurization. In other instances in which the reservoir of infection is a wild animal, immunization, when a solid immunity can be obtained, of the limited number of individuals who have contact with such infected populations is satisfactory.

The insect-borne diseases present still other problems in control in that the link between man and animal or man and man is a living organism. In general, control of the insect population or of the disease in the insect will result in control of the disease in man. The infections transmitted by insects are, for the most part, rickettsial, protozoan and virus diseases, few bacterial diseases are insect-borne, presumably because in many of the blood-sucking insects the intestinal tract is actively bactericidal and most species of bacteria are rapidly destroyed there. The mechanical transmission of bacterial infection by insects is, then, relatively rare and, except under unusual circumstances, of no great importance. Two species of pathogenic bacteria are, however, resistant to this bactericidal activity: the bacilli of plague and tularemia multiply in the rat flea (*Xenopsylla cheopis*) and the deer fly (*Chrysops discalis*) respectively. When the microorganism either multiplies or completes a portion of its life cycle in the insect vector, the insect is infective for the duration of its life; such is the case in yellow fever, typhus fever, malaria and other diseases. The problems of control of these diseases are the problems of the control of the insect population, either indirectly through the insect's animal host as in bubonic plague or directly as in malaria. A further complication is introduced in the case of spotted fever. The tick (*Dermacentor andersoni*) undergoes an incomplete metamorphosis, and the infection is "hereditary," i.e., transmitted to the second generation. There is, in consequence, not only an animal reservoir of infection but also a second reservoir in the form of infected ticks.

Diseases Transmitted Directly from Man to Man. The diseases transmitted directly from man to man by contact or by air-borne infection are by far the most difficult to control. The mechanism of transmission is, of course, very simple and does not offer the possibilities for interference that may be taken advantage of in the more complex processes. Furthermore, the practices and habits of the human population that make possible transmission by these means are a part of day-to-day existence in the life of man. Thus the aggregation of human beings under crowded living conditions in urban areas, and the theatres, schools, churches, and other public meeting places cannot be dispersed or eliminated for the control of air-borne infection. Similarly, pre-

Ingestion of the bacterium is not necessarily followed by its digestion. This is especially true of virulent forms; for example, virulent tubercle bacilli (Fell and Brieger 1947, Suter 1952) and brucellæ (Dickey and Forbus 1945) remain alive for some time inside the intact phagocyte. Rogers and Tompsett (1952) observed that, once inside the phagocytes, both virulent and avirulent staphylococci decreased in numbers, beginning 10-15 minutes after ingestion; the number of avirulent cocci remained low for 24 hours but the organisms of the virulent strain began to multiply after 4-8 hours.

Although growth of bacteria within the phagocyte in all probability leads to its death, in certain circumstances it appears that phagocytes may eject the ingested organisms in a viable state and themselves remain viable (Wilson 1953).

The continued survival of a bacterium in an intact free phagocyte, which moves in the tissues and reaches the lymph and blood vessels, may lead to the dispersion of the organisms to other parts of the body. This is not necessarily a failure of defence, as it is sometimes assumed to be, because, as we argued in Chapter 47, dispersion of the invader may have survival value for the animal, in this case by ensuring that the bacteria carried in the microphages ultimately reach the highly efficient macrophages of the reticulo-endothelial system.

The migratory power of the phagocyte, especially chemotactic migration in response to substances elaborated at the immediate site of infection, is clearly an important part of its antibacterial function, and both macrophages and microphages display this chemotropism (see Lasfargues and Delaunay 1947, Harris 1953).

These powers are affected by bacterial products. Thus, the *in vitro* migration of white blood cells into clotted plasma is inhibited by virulent, but not by avirulent tubercle bacilli; and by *Salm. typhi* and *Ps. pyocyanea*, but not by *Staph. aureus* or Type III pneumococcus. The crude endotoxins of *Salm. typhi* and *Ps. pyocyanea* also inhibit migration, but the type pneumococcal polysaccharide does not (Martin *et al.* 1950, Martin and Chaudhuri 1952). Elberg and Schneider (1953) observed a similar inhibition by brucella cells and endotoxin. Chemotactic migration also is reported as being inhibited by a lipid fraction (Allgower and Bloch 1949) and by the antigenic polysaccharide (Choucrour *et al.* 1951) of the tubercle bacillus. An adaptive response to the inhibitory effect may well take place in the infected animal, because leucocytes can be made refractory to the anti-migratory action of virulent tubercle bacilli by prior treatment of a donor animal by the organisms given intravenously (Martin *et al.* 1950), and to that of various species of *Brucella* by infection with the corresponding species (Elberg and Schneider 1953).

It remains to note some instances of non-specific increase in resistance to bacterial products. Rabbits and men, on repeated intravenous injection of small doses of enterobacterial endotoxins, acquire a tolerance to the pyrogenic effects of such doses; rabbits have also been shown to acquire tolerance to the gross toxic effects of larger doses. The tolerance, which disappears some days after the cessation of the repeated injection, is non-specific, being unrelated to the antigens in the various preparations. It is apparently due to an increased efficacy of the clearing and detoxifying mechanism of the reticulo-endothelial system, and is abolished by intravenous thorotrast and other blocking agents (Beeson 1947a, Morgan 1948, Bennett and Beeson 1953). A similar tolerance of rabbits to the provocative effects of these substances in the Shwartzman reaction (see next section) is also induced by their repeated intravenous injection.

The Bactericidal Power of the Blood.—This leads us to a consideration, necessarily brief, of the possibility of inducing an effective immunity by increasing the non-specific bactericidal mechanisms of the cells and fluids of the body. A. E. Wright

vention of the spread of venereal disease is theoretically possible but relatively unsuccessful in practice.

In the past, therefore, control of disease spread by these means has been dependent upon effective active immunization procedures. Diphtheria and scarlet fever may be so controlled by the immunization of a reasonably large part of the population, but influenza, the common cold and others continue to be widespread.

With the elucidation of the mechanisms of air-borne infection and the development of aerosols (p 150) and ultraviolet irradiation for the destruction of air-borne bacteria, it seems possible that the spread of air-borne infection may be controlled. Thus, the establishing of air sterilization in public gathering places of various kinds, possibly in the air conditioning systems, as well as in hospital wards may be effective.

The control of the spread of a given disease is, in practice, complicated by two important factors. First, a disease is often transmitted in more than one way. This variation may be within narrow limits, for example, an insect-borne disease may be transmitted by several species of insects which differ from one another in distribution, breeding habits and the like. In extreme cases a disease may fall into more than one epidemiological type; bubonic plague, for instance, which is transmitted from the rat to man by the rat flea, may assume a pneumonic form transmitted by infective droplets and become independent of the rat population. Secondly, infection may be carried and transmitted, not only by clinically recognizable cases of disease but also by cases before clinical symptoms appear; by individuals who have a disease in such a mild form that it is not recognized, and by healthy individuals who carry the infection either transiently or semipermanently. For example, while it is not difficult to prevent epidemics of water-borne typhoid fever, the disease remains endemic in a community as a result of the dissemination of the bacilli from ambulatory cases and carriers, and this so-called "residual typhoid" is extremely difficult, if not impossible, to eradicate.

It will be clear from the foregoing discussion that the transmission of infection is indeed a highly complex matter. Each disease is a special case of the host-parasite relationship which is not infrequently complicated by the interaction of two or more host populations. The elucidation of the interrelationships of these groups is a necessary preliminary to the understanding of the mechanisms operative in the dissemination of disease.

Cowan (1939) immunized rabbits with *Past. pseudotuberculosis* and found that the rate of disappearance from the blood of intravenously injected *Staph. aureus*, both immediately after injection and after establishment of the secondary bacteraemia (see p. 1191), was greater than that in normal animals, and equal to that in rabbits immunized with *Staph. aureus* vaccines. In view of the complete antigenic dissimilarity of the pasteurella and the staphylococcus, this effect is clearly non-specific. In this connection we may note that Day (1942, 1944) reported an increased immunity of mice to pneumococcal infection by inoculation with extracts of a wide variety of cocci and bacilli. Day, however, regarded the immunity as induced by a common antigen. As we saw on p. 1345, certain antibacterial mechanisms are stimulated, apparently non-specifically, during the course of naturally occurring disease in man—notably an increase in the bactericidal power of the blood associated with fever, possibly due to an increase in substances resembling β -lysin.

Taking the evidence as a whole, it would seem that non-specific stimulants of this class are for the most part too slight or too transient in their effect on resistance to be worth exploiting as prophylactics against infection. The transience of the effects is clearly no bar to their use in the therapy of established infections, where such stimuli might be beneficial, especially in the early stages of the disease; but little has been done in this direction. (The anti-infective action of hormones and ionizing radiations is discussed in Chapter 51)

The Shwartzman Phenomenon.

In the preceding paragraphs we have considered various reactions that are associated with a non-specific increase in resistance. In concluding this chapter it will be convenient to refer briefly to an experimental procedure that is associated with a localized and non-specific increase in sensitivity. Shwartzman (1923, 1929a, b, 1931, 1932, 1933) described a curious phenomenon that has become known by his name, which has been studied in considerable detail (e.g., Gratia and Linz 1931, 1932, Gross 1931, Freund 1934a, b, Freund and Smith 1934, Freund and Hosmer 1935, Morell and Shwartzman 1938, Alechinsky 1938, 1939, Weir 1938, Ayo 1943). The earlier work is fully discussed in Shwartzman's monograph (1937).

When a rabbit is injected intradermally with a small amount of a filtrate of a culture of *Salm. typhi*, and 24 hours later is injected intravenously with the same filtrate, the intravenous injection is followed, within a few hours, by the development of a hæmorrhagic lesion at the site of the intradermal injection. It is not, however, necessary that the same organism should be used for the intradermal injection and for the subsequent intravenous injection, or that the organisms used for the two injections should be antigenically related. Filtrates from, or suspensions of, a variety of bacteria will sensitize the skin to the intravenous injection of an equally wide variety of bacteria or bacterial filtrates. Not all bacteria contain effective sensitizing substances, nor is the local sensitizing effect the result of a simple inflammatory reaction. Thus (Shwartzman 1923), the injection of uninoculated culture media, of turpentine or of filtrates of various strains of streptococci, failed to sensitize the skin to the subsequent intravenous injection of filtrates of typhoid cultures. The sensitizing or, as Shwartzman prefers to call them, the "preparatory" substances are thermostable, antigenic, of relatively large particle size and often closely associated if not identical with the "complete antigens" or endotoxins of coliform and salmonella bacilli. Purified preparatory substances are said to produce no detectable change in the tissues. Not all Gram-negative bacteria contain preparatory substances (see Witebsky and Salm 1937, Wise and Kerby 1943). Nevertheless, the vascular endothelium is in some way altered by the preparations so that the intravenous "provoking" dose causes severe hæmorrhage and necrosis in the prepared site.

Chapter 10

THE BACTERIOLOGY OF WATER AND SEWAGE

Of the interrelationships between the members of human populations that facilitate the transmission of infection, those arising as a consequence of common water supplies and the group disposal of sewage are among the most important. *Transmission of disease by this means is, of course, confined to diseases of the so-called enteric group, in which infection takes place via the gastro-intestinal tract and the causative microorganisms are discharged with the feces. Infection is, therefore, the result of a direct connection between infectious fecal material and the mouth of a susceptible person, and when that connecting link is a common water supply, as it frequently is, outbreaks of cholera, typhoid fever and the like occur. This link is, however, readily broken; the great water-borne epidemics are rapidly becoming a thing of the past as a consequence of the utilization of effective control measures, and present day water-borne epidemics are indicative, not of a lack, but of a failure to make use of existing knowledge.*¹

WATER²

Because of water-borne disease and the obvious desirability of its control, studies of the bacteriology of water have been directed, for the most part, toward its sanitary aspects. The best single criterion by which the sanitary quality of a water may be judged is, clearly, the kind and numbers of bacteria that are present in it. If it were possible, as a routine procedure, invariably to detect the presence of the appropriate disease-producing bacteria, it would be unnecessary, from the sanitary point of view, to take the non-pathogenic forms into consideration. *This is, however, not the case, a judgment of the sanitary quality of a water cannot be made on the basis of failure to find a microorganism such as the typhoid bacillus in that water. This bacterium, for example, is usually so outnumbered by similar forms such as the colon bacillus that its isolation and identification require enrichment cultures in selective media and other time-consuming procedures and even then are not always successful, and a negative finding is of doubtful value.*

It is legitimate to assume, however, that when a water is polluted with human fecal material, it is probable that it contains bacteria which cause enteric infection. This probability becomes a certainty when the fecal material is pooled, as in the sewage of a community, because of the ubiquitous presence of healthy carriers of typhoid bacilli and similar microorganisms. Since it is im-

¹ Gorman and Wolman: *Jour. Amer. Water Works Assn.*, 1939, 31:225.

² Prescott, Winslow and McCrady: *Water Bacteriology*. 6th ed. John Wiley and Sons, New York. 1946.

decrease the power of leucocytes to migrate in clotted plasma. This decrease occurs in the leucopenic period after the provoking dose. Moreover, the leucocytes of Shwartzman-refractory animals are unaffected by a provoking dose (Berthrong and Cluff 1953, Cluff 1953). That the local vascular occlusion by clumped cells and fibrin is a necessary feature of the reaction is evident from the effect of heparin, which in high anticoagulant doses maintained for 4 hours after provocation, completely inhibits the reaction (Good and Thomas 1953, Cluff and Berthrong 1953). It does not affect the change in the migrating powers of the leucocytes, noted above. Thomas and Stetson (1949) suggest that local preparation may activate a protease at the site, which is held in check by protease inhibitors until the latter are destroyed by the provoking factor of toxin. They observed an analogous reaction when a provoking factor was injected one hour after an intracutaneous dose of papain (see also Christensen 1952).

The occlusion of glomerular capillaries that characterizes the generalized reaction is not cellular but hyaline, but, as in the local reaction, the participation of leucocytes in the formation of the hyaline material is suggested by its absence in nitrogen-mustard treated animals (Thomas and Good 1952).

The possibility that in natural infections there may be local alterations in the reactivity of tissues, particularly of vascular tissues, induced non-specifically by substances of bacterial origin, cannot be overlooked in our attempts to analyse the phenomena of resistance to infection. Thus, the hæmorrhagic episodes in certain chronic infections of man may be related to the phenomenon (see Shwartzman *et al.* 1936). There is ample ground for speculation, but little direct proof.

Local infections with a variety of bacteria—*e.g.* streptococci and the tubercle, anthrax and influenza bacilli—are preparatory in the Shwartzman sense. A general infection with Group A streptococci prepares for a generalized reaction, but *in vitro* streptococcal cultures, or parts thereof, are not preparatory (Thomas *et al.* 1953). However, extracts of streptococcal skin lesions from the rabbit will prepare normal rabbits, so that a Shwartzman-like reaction can subsequently be provoked by streptococcal culture filtrates rich in streptolysin O (Schwab *et al.* 1953). As we have seen, some pathogens contain both preparing and provoking factors; these possibly operate as such in natural infection, to the detriment of the infected animal. For example, Cohen and Moolten (1943) found both factors in a virulent strain of *F. necrophorus*, but preparatory factors only in an avirulent strain. In rabbits infected intratracheally with the latter, the intravenous injection of provocative factors, derived either from the virulent strain or from the meningococcus, precipitated a fatal infection.

The generalized Shwartzman reaction may be inadvertently elicited in man; thus, Love and Driscoll (1945) report a fatality following two injections of a typhoid vaccine separated by a 24-hour interval. But aside from a possible connection between hæmorrhagic phenomena in certain chronic infections in man (see Shwartzman, Klemperer and Gerber 1936) it is at present impossible to say what part this mechanism plays in natural infection and resistance, or in artificial immunization.

SUMMARY

(1) In addition to the specific factors that determine antibacterial immunity, there are certain non-specific factors which appear to play some part in the reaction of the tissues to invading bacteria. The significance of these factors in general defence is difficult to assess.

(2) The blood plasma in certain animal species contains antibacterial substances, usually thermostable, of which the β -lysinins are an example. Their bactericidal action is limited to particular bacterial species, and it seems doubtful whether they confer an effective immunity even against these. They are not

practical to isolate these pathogenic bacteria themselves as a routine measure, some indicator of fecal pollution will serve equally well as a criterion of the sanitary quality of a water. The question, which was stated many years ago,³ becomes this: Are there types of bacteria which are never present in natural waters free of pathogenic bacteria but which may always be found in water polluted with human fecal material and therefore probably with pathogenic bacteria? If so such types of bacteria can be used as indicators of pollution. The answer to this question involves a consideration of the bacterial flora of natural waters, including both bacteria which are native inhabitants of water and those microorganisms whose presence is a consequence of contamination from external sources.

Bacteria Native to Natural Waters. The bacteria whose native habitat is water are not well known,⁴ in part because many of them are difficult to grow on laboratory media. There is no question, however, of the existence of a bacterial flora normal to and characteristic of natural waters. The types of bacteria which make up this natural population may be considered briefly:

- (1) *higher bacteria*, frequently the sheathed forms assigned to the *Chlamydo-bacteriales* and including sulfur, iron and other forms,
- (2) the *Caulobacteria*, a relatively recently described group of "stem" bacteria which occur in lakes and other bodies of water attached to some inanimate object
- (3) the *spiral forms*, which are frequently found in great numbers in water, some of which may be very large, 20 to 30 μ in length, as compared with the parasitic spirilla:
- (4) a variety of *bacilli*, including
 - (a) pigmented forms such as *Bacterium prodigiosum*, *Bacterium violaceum*, *Bacterium aureum*, and others,
 - (b) various non pigmented forms such as
 - 1 the fluorescent bacteria—*Pseudomonas fluorescens*,
 - 2 certain of the sulfur bacteria,
 - 3 thermophiles,
 - 4 aerobic, spore forming bacilli of uncertain taxonomic position,
- (5) *coccus forms*, both
 - (a) pigmented, generally yellow—very often *Sarcina lutea*, and
 - (b) non pigmented—*Micrococcus aquatilis*, *Micrococcus caudicans*, and others,
- (6) *nitrogen fixing bacteria*—*Azotobacter aquatilis* in particular.
- (7) *nitrifying bacteria*—both *Nitrosomonas* and *Nitrobacter*.

These water bacteria are found in fresh water in swamps, streams and lakes. The bacterial populations of salt waters have not been studied until recent years, but it appears⁵ that the sea contains similar bacteria, including the nitrogen fixing forms, the fluorescent bacteria, various pigmented forms and

³ Cf. Jordan in Thirtieth and Thirty first Repts. Mass. State Bd. of Health, 1898, 1899.

⁴ Cf. the review by Bauer. *Studien zur Hydrobakteriologie stehenden Binnengewässer* Arch. f. Hydrobiol., 1935, 29:183, also the studies of Taylor. *Jour. Hyg.*, 1940, 40:616, *ibid.*, 1941, 41:17, *ibid.*, 1942, 24:284.

⁵ Zöllbel and Feltham. *Bull. Scripps Inst. Oceanog., Tech. Series*, 1934, 3:275, Waksman, Hotchkiss, Carey and Hardman. *Jour. Bact.*, 1938, 35:477, Zöllbel. *Marine Microbiology*. Chronica Botanica, Waltham, Mass. 1946.

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like, and each instance of contamination or possible contamination must be considered individually. Fecal bacteria have been found to penetrate from 100 to 200 feet in ground water.⁸ In general fine soils and sand tend to impede their progress to a greater extent than coarse sand and gravel. The rock formations may be of considerable significance; sandstone, for example, filters out bacteria, while limestone tends to erode with the formation of direct communicating channels, and wells drilled into such formations may contain typhoid bacilli which entered the water many miles away and are, therefore, always to be regarded as dangerous.

Contamination by Human Excreta. Contamination of water by human excreta may take place, not only indirectly through the agency of soil as noted above, but also directly. Such direct contamination is, for the most part, a consequence of human population densities and urban organization, and takes the form of dumping of sewage of one community into a body of water which serves as a water supply to another. Whether directly or indirectly contaminated, such waters contain not only the native bacterial flora supplemented by microorganisms from the soil, but also the bacterial flora of the human intestine. The contribution of the last consists primarily of *Bacterium coli* in very large numbers, together with *Clostridium welchii*, *Streptococcus fecalis* and the various intestinal pathogens.

Factors Influencing the Kinds and Numbers of Bacteria. The numbers of bacteria that may be found in a given water are dependent primarily upon the type of water, whether it is a surface water, such as that found in streams, lakes and shallow wells, or a deep water from deep driven wells. In the first instance opportunities for contamination are great and, as might be expected, many bacteria are present. The water from deep wells, on the other hand, has undergone an effective filtration in order to reach the deeper strata in which it is obviously not subject to any extensive contamination; hence relatively few bacteria are found.

A variety of environmental factors influences the bacterial content of water; chief among these are the amount of organic matter present and the temperature. In general, the more nutriment there is present in the form of organic matter, the greater the number of bacteria. Low temperatures are not conducive to rapid growth and tend to keep the numbers of bacteria down, a factor that favors the survival of pathogens such as the typhoid bacillus which are unable to multiply in any case. Higher temperatures result in an increase in bacterial numbers in the presence of sufficient organic matter, but if the supply of nutriment is not great, after a preliminary increase during which the food supply is exhausted, the numbers fall below the initial level. Other environmental factors are not infrequently influential in determining the types of bacteria present in a water; thermophiles will, of course, predominate in hot springs and sulfur bacteria in sulfur springs; and the acidity of many natural waters results in a limited flora of acid-resistant bacteria.

Bacteria in Ice.⁹ Although it is difficult if not impossible to sterilize a substance by exposure to low temperatures, many of the bacteria present are killed, only the resistant cells surviving. The great majority of bacteria in water are

⁸ See, for example, the studies of Caldwell Jour. Inf. Dis., 1938, 62:225, 272

⁹ See Jenson: Food Research, 1943, 8:265.

CHAPTER 53

LOCAL IMMUNITY

THE defence mechanisms with which we have hitherto been concerned have for the most part been generally effective throughout the tissues of the host; and by whatever route bacteria or their products gain access to the tissues these mechanisms will tend to protect the body as a whole.

Another type of immunity may be conceived, confined to one particular area of the body, or to one particular kind of tissue. In Chapter 45 we have discussed certain mechanisms that hinder the access of bacteria to the tissues, many of which, like the lysozyme in the conjunctival sac, or the acidity of the gastric juice, are functions of a particular locality in the body. We may distinguish these from the mechanisms of local immunity which we consider in this chapter, by assuming that they act on the infecting organism before it gains its primary lodgment in the tissues. It will be clear, nevertheless, that the distinction, though convenient, is arbitrary, for the hindering mechanisms may in fact be located in the tissues; and even if their action, like that of gastric juice, takes place manifestly outside the tissues, the effect is ultimately dependent on local tissue function. We must, therefore, be prepared if necessary to accept a superficial hindering mechanism of this kind as an expression of a true local immunity, though we shall in the meantime confine ourselves to manifestations of resistance within the tissues.

An enhanced local resistance may or may not be associated with a significant increase in the resistance of the body as a whole; moreover, it may depend on a local variation in the response of a generalized defence mechanism; or upon the reaction of special tissues or types of cell in the area under consideration. There has been a tendency to confuse the issue of local versus general immunity by inadequate definition of the problem to be solved. The concept of a local immunity as such has been imperfectly separated from hypotheses regarding the mechanism on which it depends. In some cases the term has been used as though it implied a tissue or cellular immunity, not conditioned by humoral factors. In others there has been a failure to distinguish clearly between specific and non-specific effect. It will simplify discussion if we consider the available evidence from each point of view in turn.

What evidence is there for the existence of a local as opposed to a general immunity

As long ago as 1887 Meierowitsch (1888) noted that rabbits which had recovered from a local infection were not susceptible to a subsequent general infection confirmed by many later workers, and Gromakowsky (1899) noted that

killed by freezing. Hence ice always contains but a fraction of the number in the water from which it was formed. Over 90 per cent both of the ordinary water bacteria and of typhoid bacilli die within a few hours, and a progressive decline in numbers then takes place, less than 1 per cent of typhoid bacilli surviving at the end of a week of freezing. Ice stored for six months is practically sterile. Outbreaks of typhoid fever have rarely been traced to the use of ice, although in a few instances the evidence of ice transmission seems quite conclusive. Danger of typhoid infection from the use of ice in drinking water is, in the absence of direct contamination of the ice during handling, always less than from the use of water from the same source as the ice.

The Bacteriological Analysis of Water.¹⁰ It will be apparent from the above discussion that the bacteria whose presence in water is a consequence of fecal pollution are not present in uncontaminated water and are sufficiently different from the native water bacteria that they may be readily distinguished. Of these bacteria *Bacterium coli* is present in greatest numbers, while *Streptococcus fecalis* and *Clostridium welchii*, although constantly present, are usually not so numerous. It would appear, therefore, that any of these microorganisms could be used as an indicator of pollution. Of these *Bact. coli* is the most satisfactory, although both *Str. fecalis* and *Cl. welchii* have been used in Europe. The streptococci are, however, sometimes difficult to differentiate and die out more rapidly than coliform bacteria. *Cl. welchii* has the disadvantage that its spores remain viable over long periods of time in contrast to *Bact. coli* which, although more hardy than the typhoid bacillus, dies out in time, hence *Cl. welchii* does not allow the differentiation of recent and old pollution. The quantitative relations between the coliform bacteria and the enteric pathogens are discussed at some length by Kehr and Butterfield.¹¹

The bacteriological examination of water for the presence of *Bact. coli* rests upon the fact that this microorganism ferments lactose. The standard procedure for the examination has been prepared jointly by the American Public Health Association and the American Water Works Association, and is revised at frequent intervals.¹² Briefly, it consists of three parts, (1) the presumptive test, (2) the confirmed test and (3) the completed test. In the first, lactose broth is inoculated with decimal dilutions of the water sample, commonly 10 ml., 1 ml. and 0.1 ml. (expressed as dilutions these are, respectively, 0.1, 1 and 10). The volume of the smallest inoculum producing fermentation provides a crude approximation of the numbers of *Bact. coli* present in the water. Of the selective enrichment media, containing ox bile and/or brilliant green or ricinoleate or lauryl sulfate, only lauryl sulfate tryptose broth has been officially accepted for the presumptive test without confirmation, and then not for filtered or treated waters. A more accurate estimate may be obtained by inoculating five tubes with each dilution and calculating the most probable number of *Bact. coli* on the basis of the number of tubes in which

¹⁰For British practice, which differs slightly from American, see Ministry of Health Rept. No. 71, *The Bacteriological Examination of Water Supplies*. His Majesty's Stationery Office, London, 1939.

¹¹Kehr and Butterfield. Pub. Health Rep., 1943, 58-589.

¹²This procedure may be found in detail in *Standard Methods of Water Analysis*, American Public Health Association, 9th ed., 1946, and in condensed form in most of the standard laboratory manuals.

We need not doubt that a local immunity exists, in the sense that one area of the tissues is more resistant than another to the consequences of a primary lodgment of a living bacterium or virus. The problem at issue is whether these *localized* differences in resistance can be accounted for by local variations in the effectiveness of the mechanisms we have considered in previous chapters, or whether some essentially different mechanism is operative.

Is the distribution of a local immunity confined to a particular type of cell or tissue, affecting that type of cell or tissue wherever it occurs in the body, or is this distribution determined by the site of inoculation, affecting all cells or tissues in the immediate neighbourhood of that site?

Continuing with the example of erysipelas, we may inquire whether it is the skin, as such and apart from other tissues, that is rendered resistant by the intracutaneous injection of living hæmolytic streptococci, or whether the effect is limited to the area of the initial erysipelatous reaction, affecting in that area all the cells or tissues involved in the inflammatory process.

The evidence is somewhat conflicting. The observations of Gay and Rhodes are consistent with the immunization of the skin as a whole, as the result of a localized experimental erysipelas. The findings of most other workers suggest that the distribution of resistance is, in the first instance, confined to the area affected by the erysipelatous reaction, though other areas of skin share in the more general immunity that may be induced by repeated injections of streptococci. As Amoss and Bliss showed, outside the area of erysipelatous reaction, the skin showing the greatest increase in immunity was that lying in the path of drainage through the underlying lymphatic plexus. The point is of some importance in deciding whether the increased immunity displayed by the skin as a whole results from immunization of the skin as an organ, or is only a manifestation of a high general immunity.

Landsteiner and Chase (1939) recorded some experiments which are relevant to the issue. They were able to sensitize the entire skin of a guinea-pig to poison ivy by local application of an extract to one area of it. Isolation of the area of application, by removing an annular piece of skin so as to leave an island, did not impair general sensitization even though the whole thickness of the skin was thus removed. When the underlying lymphatic plexus, and consequently the only pathway for the ready absorption of the extract into the body as a whole was severed, general sensitization did not follow application of the extract.

The question posed leads naturally to the one that follows it, and we shall consider the evidence relevant to its solution under this and other heads

Does the local immunity as such, and apart from an associated humoral immunity, confer an increased resistance on the body as a whole?

It will be convenient to commence our discussion of this question by setting out, in their baldest terms, the views that were put forward by Besredka (1919, 1920, 1921)

He noted that many, if not all, pathogenic bacteria tend to localize in particular tissues of the body. Thus the typhoid, paratyphoid and dysentery bacilli cause intestinal infection, and the anthrax bacillus commonly causes infection of the skin. However,

fermentation occurs. Tables for the calculation of the most probable numbers are given by Prescott, Winslow and McCrady.² The *confirmed test* consists of the inoculation of a specified selective medium such as Endo or eosin-methylene blue (EMB) plates, brilliant green lactose bile broth, crystal violet lactose broth, fuchsin lactose broth or formate ricinoleate broth. The appearance of typical *coli* colonies on the plates or fermentation in the selective lactose broth constitutes a positive confirmed test. In the *completed test* one or more typical colonies are picked from an Endo or EMB plate inoculated either from the original lactose broth culture or from the secondary selective medium showing fermentation and are transferred to an agar slant and a lactose fermentation tube. After incubation the slant culture is smeared and stained and examined for the gram-negative non-spore-forming rods of *Bact. coli*. If the culture is found to be morphologically *Bact. coli* and the lactose is fermented, the completed test is positive.

The Coliform Bacteria. Although *Bact. coli* is readily distinguished from the native water bacteria, the closely related *Bacterium aerogenes* is found in grains and elsewhere in nature though not, it may be noted, in virgin soils. The two bacterial species may be differentiated by a number of tests (p. 422), but these are not a part of the standard method of water examination; hence the gram-negative, lactose-fermenting bacteria whose presence is determined are more properly termed *coliform bacteria* rather than *Bact. coli*.

The Sanitary Significance of Coliform Bacteria. In recent years there has been a tendency in sanitary water analysis to emphasize the distinction between *Bact. coli* and *Bact. aerogenes*, the presence of the latter being considered by many to have little or no sanitary significance. There is no doubt that *Bact. coli* is more predominantly of "fecal origin" while *Bact. aerogenes* is found in greater relative abundance in soil than in sewage. For this reason it is maintained that a predominance of *Bact. aerogenes* over *Bact. coli* in a water supply is more indicative of soil contamination (the *coli* presumably of animal origin) or past pollution than of recent pollution. In general, however, while the proportion of *Bact. coli* to *Bact. aerogenes* is frequently correlated with the sanitary survey, there are too many exceptions to warrant attaching great significance to it. The ecology and significance of the different types of coliform bacteria found in water are reviewed and considered in detail by Taylor.¹³

It should be remembered, however, that coliform bacteria of all kinds are practically absent from virgin soils and from pure spring and surface waters, and that while *Bact. aerogenes* and intermediate forms are not present in feces in as great numbers as *Bact. coli*, their presence may nevertheless be demonstrated by appropriate methods.¹⁴ Even if it is true that *Bact. aerogenes* is somewhat more resistant than *Bact. coli*, and hence may survive in soil or water long after the latter has disappeared, the fact that its presence is not reliable evidence of *recent* pollution may not be as decisive as sometimes assumed. Experience in water examination has shown that it is not safe to disregard the warning of potential danger conveyed by evidence of soil washings and "past pollutions." It is unwise, therefore, in routine water analysis, to place

¹³ Taylor Jour. Hyg., 1942, 42:23.

¹⁴ Gray Jour. Hyg., 1932, 32:132; Bardsley: Jour. Hyg., 1934, 34:38

concluded that the rabbit can resist a large subcutaneous inoculation of *B. anthracis*, provided that the bacilli do not come into contact with any wound of the skin.

Other investigators have, however, recorded quite different results. Sobernheim and Murata (1924) estimated the minimal lethal dose of a culture of *B. anthracis* when administered by different routes. They found that about 1/10,000 of a loopful would produce death when introduced intraperitoneally or intravenously, about 1/100,000 of a loopful when introduced subcutaneously or intracutaneously and about 1/1,000,000 of a loopful when injected intramuscularly.

It is obviously difficult to avoid infecting the skin when making injections into any subjacent tissue, and this objection has been urged against those who have recorded successful infection by some route other than the intracutaneous; but Besredka's assumption can hardly be reconciled with the observation that a smaller dose of bacilli is required to produce a fatal infection when the inoculation is made into the muscles than when it is made directly into the skin. Similarly, Basset (1925) was unable to demonstrate any superiority of the intracutaneous as compared with the subcutaneous or intramuscular route. (See also p. 1185.) Müller's (1925) results are opposed to those of Plotz. Finding that anthrax bacilli would grow slowly through the pores of an L_1 candle, he placed cultures of the organism in such candles, sealed them carefully, and introduced them into the peritoneal cavities of nine rabbits. Seven of these animals died after intervals varying from 6 to 51 days. This long incubation period would appear to preclude an infection of the skin occurring at the time of the introduction of the candles. Burke and Barnes (1931) exploited the bactericidal action of gentian violet in an attempt to produce an initial subcutaneous infection with *B. anthracis* unassociated with any primary infection of the skin. A guinea-pig was injected with a young culture of *B. anthracis* by introducing the needle through the skin of the abdominal wall in one flank, and then passing it subcutaneously across the abdomen to the other flank, where the inoculum was deposited. The point of the needle was then withdrawn to just beneath the skin puncture, the syringe barrel was detached, another barrel containing a 1 per cent. solution of gentian violet was attached, and the dye was introduced into the puncture as the needle was withdrawn. The guinea-pig died in 56 to 72 hours. The characteristic gelatinous infiltration occurred at the point where the inoculum had been deposited, not at the point where the needle had been inserted. Cultures from the dye-stained area about the site of the skin puncture remained sterile.

Clearly Besredka's contention with regard to the unique susceptibility of the skin to anthrax has not been proved. Besredka and his colleagues (Besredka 1924*a, b*, Besredka and Urbain 1924) also attempted to immunize the skin against other bacteria. They applied filtrates from broth cultures of staphylococci or streptococci to the shaved skin of rabbits, and tested the resistance of these areas of skin to subsequent infection with the homologous organisms. They concluded that this procedure conferred a high degree of local immunity. In later reports they, and others, recorded valuable curative properties in these filtered cultures—the so-called "antivirus" preparations—but these observations do not bear directly on the problem at issue, and need not be further considered here.

We need not doubt that various procedures of this kind may raise the resistance of local areas of skin to subsequent infection; but it by no means follows that this is associated with an increase in the resistance of the body as a whole.

Thus, Hach, Borodaj and Melnyk (1928) state that staphylococci, after intravenous inoculation into rabbits, tend first (3-3½ hours) to accumulate in the spleen, few remaining in the blood and few being present in the skin. As the infection progresses towards its rapidly fatal issue the distribution changes; the number of cocci in the spleen rapidly diminish, the number present in the skin show an enormous increase. Multiple intradermal inoculations of a staphylococcal filtrate, preceding the intravenous injection, prevented this rapid accumulation of staphylococci in the skin (Hach and Melnyk 1928); but there was no effective immunity, the rabbits dying of typical acute staphylococcal infection.

too much stress upon the differentiation between *Bact. aerogenes* and *Bact. coli*, at all events until the practical value of such differentiation can be clearly demonstrated.

Plate Counts. It is usually desirable to have an approximate measure of the total number of bacteria in drinking water, not because the sanitary quality of a water can be judged on this basis alone, but because such information frequently has ancillary value. The counts obtained by quantitative dilution and plating are, it must be remembered, those of the microorganisms that will grow on the medium used, other bacteria being quite inapparent by this method.

Two series of plates are poured, one in which the medium is nutrient gelatin and the other nutrient agar, or both may be nutrient agar. The gelatin plates, or one set of agar plates, are incubated at 20° C. and the agar plates at 37° C.¹² In general the native water and soil bacteria grow best at 20° C.; in some cases they do not grow at all at 37° C.; and bacteria of animal origin grow most rapidly at body temperature. The relative numbers of microorganisms growing at the two temperatures are, then, at times suggestive of the origin of the bacteria found.

Chemical Analysis. The analysis for appropriate chemical compounds frequently is of value as an adjunct to bacteriological analysis in the determination of the sanitary quality of a water.¹² Pollution by sewage, for example, adds complex compounds, protein, carbohydrate and fat, to the water, and the amount and state of the decomposition products of these substances may serve as an index of the degree and time of pollution. Ammonia, nitrites, nitrates, chlorides and albuminoid nitrogen are usually determined. Of these, chloride, and to a certain extent nitrate, are the most useful. It may be noted that the chemical analysis of water is frequently made in connection with hardness, turbidity, taste, smell and similar features, which, while often of considerable industrial or esthetic significance, are of no sanitary importance.

The Assay of the Sanitary Quality of Water.¹³ The means by which the sanitary quality of a water is judged may be summarized briefly

- (1) The bacteriological analysis, including both
 - (a) the presence or absence of coliform bacteria and
 - (b) the number and type of bacteria present,
- (2) the type of water, whether surface or deep,
- (3) the local conditions, and
- (4) chemical analysis.

Of these the presence and numbers of coliform bacteria are the most important, and it must be remembered that the relative abundance, rather than the presence, of these microorganisms is the essential feature of the test. The discovery of a single colon bacillus in 50 ml. of water, or even occasionally in 5 ml., affords no reasonable ground for suspicion of the water. The possibility of sporadic contamination with colon bacilli derived not from man but from domestic animals or birds must be kept in mind. Manured fields and pastures, filled with grazing cattle or sheep, are likely sources of colon bacilli and may give rise to mistaken inferences if the environmental examination of a water supply is

¹³ Streeter: *Laboratory Control of Water Supplies* Public Health Reports, Suppl. No 201, 1948

Gay and Morrison (1923) injected various substances intraperitoneally into rabbits, and injected living streptococci 24 hours later. They found that certain substances, such as meat-extract broth, afforded considerable protection, whereas others, such as aleuronat, did not. They noted that the substances conferring protection were those that produced exudates in which macrophages were particularly numerous; on the other hand those that produced exudates containing numerous polymorphonuclear cells but few macrophages were far less effective.

Oerskov and Kauffmann (1936) and Oerskov (1940) recorded examples in mice of an intraperitoneal immunity to Vi forms of *Salm. typhi* and to *Sh. dysenteriae* that could be induced non-specifically by the injection of living or dead preparations of bacteria having no antigenic relationship to the infecting strain. Philipson (1937) made an extensive study of the immunity to *Sh. dysenteriae* induced in mice by vaccines of *Salm. paratyphi B*. A relatively high degree of non-specific immunity to intraperitoneal *Sh. dysenteriae* was demonstrable 8 hours after an intraperitoneal dose of vaccine, and lasted for 3-9 days. When two doses of vaccine were given at 9 days' interval, the immunity was demonstrable 2 hours after the second dose, and lasted up to 21 days. Thus the period of induction of the immunity was shortened, and its duration lengthened, by the re-vaccination. An intraperitoneal immunity of this degree, demonstrable 2 hours after the second dose of vaccine, could not be induced by intravenous vaccination and re-vaccination, nor by intravenous vaccination and intraperitoneal re-vaccination. Some immunity, however, followed intraperitoneal vaccination and intravenous re-vaccination, indicating that the stimulating effect of re-vaccination could be elicited in the peritoneum by blood-borne vaccine. Though the immunity was clearly non-specific, the stimulating effect of re-vaccination was in some way dependent on specific factors, for vaccination by one species of bacterium, and re-vaccination by an antigenically unrelated bacterium, was not followed by any significant increase in immunity.

Walsh and Cannon (1936) immunized rabbits against intranasal infection of the lungs with Type I pneumococci, by five or more daily instillations of formol-killed Type I pneumococci. The immunity was specific, though it appeared before antibodies were demonstrable in the blood of the animals. Cannon and Walsh (1937) recorded experiments which point to the induction of a local, perhaps specific, immunity in the lung by this procedure—an immunity apparently dependent on an increased resistance to the passage of the cocci from the alveoli into the tissues (see also Exchaquet 1936). Similar results were obtained by North and Anderson (1942), who found that intranasal *H. pertussis* vaccines were more effective than intraperitoneal vaccines for immunizing against intranasal *H. pertussis*. They concluded, however, that the superiority of the intranasal route was due to non-specific immunity, since the antibody response to vaccination by either route was similar. Intranasal vaccines made of organisms more or less related to *H. pertussis*—*H. influenza*, *H. parapertussis* and *H. bronchisepticus*—were also effective. No immunity resulted from instillation of staphylococci or pneumococci, but a *Bact. coli* vaccine was highly effective. The efficacy of the *Bact. coli* appeared to depend on its capacity to induce a histological reaction in the lung similar to that induced by *H. pertussis*. Serologically speaking, the effect was non-specific. It is salutary to reflect that, had the test with *Bact. coli* been omitted, the evidence would have been of the kind we are accustomed to regard as a reasonable indication of the specificity of a particular immunizing effect (see also Gray 1947, Cooper 1952).

Pullinger (1936, 1938) provided an instructive example of a clear-cut increase in non-specific local immunity dependent on the mobilization of cells in the lymph nodes of the guinea-pig. The generalized infection following an intramuscular injection of *Br. abortus* was largely inhibited when the organisms were injected together with a second bacterium, *Myco. tuberculosis* or *Listeria monocytogenes*. Injection of the second bacterium into another site did not affect the brucella infection, except to a very slight extent in animals receiving the tubercle bacillus; nor did the second bacterium act as an adjuvant, improving the animal's antibody response to the brucella. The effective immunization appeared

neglected. Knowledge of such local conditions as well as the type of water is, then, essential to the interpretation of the bacteriological findings. Chemical analysis may be of considerable help in some instances, but in general finds its greatest utility in the study and control of gross pollution in which the decomposition of organic matter and presence of industrial wastes are a nuisance, rather than the assay of the purely sanitary quality of a water.

Drinking Waters. The question of the significance of the bacteriological findings brings up the matter of standards to which a water suitable for drinking should conform. Now it will be clear that, although considerable numbers of colon bacilli in a water are always suggestive of fecal contamination by man or animals, a standard is necessarily a minimum which is inherently difficult to define under the circumstances. In this country the United States Public Health Service has prepared recommended standards¹⁶ which are intended to represent a minimum. In the recommended procedure the frequency of sampling is dependent upon local conditions, the minimum varying from one per month for a population of 2500, to 500 per month for a population of 5 million. The sample is 5 10 ml. portions, or 5 100 ml. portions. The results of bacteriological examination by Standard Methods procedures should be as follows

- (1) Of all 10 ml. portions examined per month, not more than 10 per cent shall show the presence of coliform bacteria.
- (2) Occasionally more than 3 of the 5 samples show coliforms, this must not occur in more than 5 per cent of samples when 20 or more samples are taken per month, or in more than 1 sample if less than 20 samples are taken per month.
- (3) Should such a result (as in 2) be obtained from a single standard sample, daily testing must be carried out until at least 2 consecutive satisfactory samples have been found. Such daily samples are to be regarded as "special samples" and not included in the monthly totals.
- (4) With regard to the 100 ml. samples:
 - (a) Not more than 60 per cent shall show coliforms.
 - (b) Occasionally all 5 portions constituting a single sample will show coliforms, this must not occur in more than 20 per cent of samples when 5 or more samples are examined per month, or in more than 1 if less than 5 samples are taken per month. If so, daily "special samples" must be taken as above.

The water shall be satisfactory as to taste, odor and color and shall contain less than the following chemical impurities: lead, 0.1 ppm, fluorine, 1.5 ppm, copper, 0.3 ppm, iron and manganese, 0.3 ppm; magnesium, chloride and sulfate, 250 ppm, total solids, 500 ppm phenol, 0.001 ppm.

The British Ministry of Health suggests standards¹⁷ based on the presumptive coliform count as determined by acid and gas formation in MacConkey broth on the piped supply entering the distribution system. Waters are divided into classes on the following basis.

Class I—Water of class I and regarded as highly satisfactory contains less than 1 coliform per 100 ml.

¹⁶ United States Public Health Service: *Manual of Recommended Water Sanitation Practice*. Pub. Health Bull. No. 296, 1946. These standards are also summarized in *Pub. Health Rep.*, 1946, 61:371; and in *Jour. Amer. Water Works Assn.*, 1946, 38 361.

¹⁷ *The Bacteriological Examination of Water Supplies*. Ministry of Health Series No. 71. His Majesty's Stationery Office, London. 1939.

acquire a specifically altered reactivity in this way. Work on the transference of the delayed type of hypersensitivity provides an example of an even more profound specific modification of cells, in this case the lymphocytes. These examples of specific immunological changes relate to hypersensitiveness; in the field of immunity proper, we have Lurie's demonstration (p. 1197) of an acquired apparently *cellular* immunity to the tubercle bacillus as a model for the kind of property we are discussing.

Little is known about non-specific immunity at the cellular level. As we saw in Chapter 53, the distribution of infective lesions among the various organs of the tuberculous animal suggests an organ resistance which may be referable to some antibacterial substance peculiar to, or in abundance in, the cells of that organ. Equally characteristic, though different, distributions occur in other bacteræmic infections, as reference to various bacterial pathogens in Parts II and IV will show.

The freedom of a given organ from lesions due to blood-borne infection can, however, be no more than suggestive of an organ immunity based on peculiarities of the cells of that organ, until alternative determining factors, like the anatomical relations of the organ to the blood stream, are eliminated.

SUMMARY

The evidence available appears to justify the following conclusions.

(1) By the injection of various materials into the tissues, and by certain analogous procedures, it is possible to induce an immunity which is confined to the neighbourhood of the treated area, and is not shared by the body as a whole.

(2) This immunity appears to be distributed in accordance with the area involved in the original inflammatory reaction, *not in accordance with the distribution throughout the affected part or throughout the body generally of one particular type of cell.* There is good evidence of a localized immunity affecting a *region* of skin, or a particular serous sac. There is no adequate evidence of an immunity affecting the skin as a whole, or the intestinal mucosa as a whole, or serous surfaces as a whole, except as the expression of a general immunity.

(3) It follows that we cannot accept the conclusion that a localized immunity of the latter type is an essential factor in a general immunity. Even had an induced skin immunity, or mucous membrane immunity, been shown to exist, our knowledge of the general defence mechanisms of the body would prevent us from regarding such a local increase in resistance as the essential factor in antibacterial immunity.

(4) All the evidence suggests that non-specific factors are of primary importance in those instances of local immunity that have been submitted to experimental study. It would appear to be the initial inflammatory reaction as such, and the cellular changes which persist for some time after it has subsided, that determine the relative resistance of the treated area to subsequent experimental infections. In particular it would seem that any treatment which induces a local mobilization and concentration of histiocytes will confer on the treated area an increased resistance, which will last as long as the local cellular changes persist. There is some evidence that with the decline of the cellular change, the treated area remains for some time more responsive than normal to non-specific immunization.

A
class I, not more than 80 per cent below class II, and none below class III.

While a presumptive test alone is specified, the occurrence of positive presumptive tests has been found to be very high in Britain. Waters of classes I and II conform closely to the American standards.

Swimming Pools and Bathing Places. The sanitary control of water in swimming pools and bathing beaches is similarly based on bacteriological examination. The American Public Health Association has recommended that not more than 15 per cent of samples of swimming pool water contain more than 200 bacteria per ml. or give positive confirmed tests for coliforms in any of 5-10 ml. samples when the pool is in use. The standard is as high as that for drinking water, but pools are usually filled with water of drinking quality and pollution is not only derived from bathers but is fresh and may be highly infective. The presence of acid forming streptococci is also of considerable utility as a measure of oral and skin contamination, and usually corresponds closely to the total count. Such waters commonly contain residual chlorine which must be neutralized with thiosulfate when samples are collected for bacteriological examination. In natural outdoor bathing places the test for coliforms is the most important. The standards are necessarily much more lenient than those for indoor pools, and those adopted locally vary from an allowable 100 coliforms per 100 ml. in California and Indiana to 3000 coliforms per 100 ml. allowed by the New York City Health Department.

The Purification of Water Supplies. When, by bacteriological examination or otherwise, a water is known to be unsafe for consumption, the question arises as to ways and means of artificial purification. There are a number of useful methods of purifying water, differing according to the amount and character of the water to be treated, which may be summarized as follows:

(1) mechanical methods

(a) storage

(b) filtration

1. slow sand filtration

2. coagulation and rapid sand filtration

(2) chemical methods

Of these methods, storage and slow sand filtration are the simplest and most economical. They are, however, not always applicable. In some cases, particularly in the purification of impounded waters because of the exhaustion of the food supply and the consequent death of bacteria and settling, not so much of bacteria alone as of suspended matter which carries down bacteria with it. The partial removal of suspended matter is frequently desirable, particularly with turbid waters, and may be carried out by allowing the water to remain in a settling basin for a time.

Slow sand filtration is one of the earliest and most effective methods of water purification and is in use in many European and some of the older American

CHAPTER 54

THE INFLUENCE OF DIET, FATIGUE, CHANGES IN TEMPERATURE AND HUMIDITY, CHEMICAL AND CHEMOTHERAPEUTIC AGENTS AND OTHER FACTORS ON GENERAL OR LOCAL IMMUNITY

The Influence of Diet on Immunity

THE traditional association of famine and pestilence epitomizes an age-old belief in the influence of diet on resistance to infective disease. Modern epidemiology and social medicine have substantiated that belief by establishing associations between malnutrition and certain infective diseases—such as tuberculosis. Malnutrition itself, however, is in turn linked with so many interrelated and variable factors in the human environment, and the human herd is so ill-suited to the kind of controlled experiment that would enable us to estimate the importance of these other factors, that there are few instances where the association could with complete confidence be interpreted as a causal relation.

To establish that relation we have at present to rely almost entirely on animal experiment. Our success in the field will depend firstly on how far the defence mechanisms that are susceptible to investigation adequately represent the processes likely to be influenced by diet; and secondly on how far we can identify the effective components of the diet, and their relation to one another. We must consider the food as a source of energy, of nutrients, and of accessory food factors. We shall find many reports on the action of dietary factors to be contradictory; in some cases, doubtless because the components of the diets were heterogeneous, and in others because in each experiment different variables were controlled.

We discussed in Chapter 43 some of the variables that affect the measurement of resistance; for example, the genetic constitution, age, sex, and methods of maintenance of the experimental animals. The commonest dietary test consists in depriving the animal of a food; but such deprivation means little unless we know such things as the initial nutritional state of the animal, how long the deprivation should be maintained to ensure that the animal's tissue reserves of the food in question are depleted, and whether the food is not being supplied, say, by the microbial flora of the gut (see Chapter 49). When the animal is depleted, the observed changes in resistance may be due, not to the lack of one factor, but to the presence of another whose ill-effects in the full diet were antagonized by the factor we have removed. Again, the depletion may have caused a loss of appetite, and so led to an effective deficiency of a factor in the diet on which the animals are being maintained. These ambiguities can sometimes be avoided by proper design of the test. In the design, we must also decide what kind of resistance to measure. If, for example, we infect the animal by natural means—such as the oral route in mouse typhoid—the subsequent morbidity rates will measure resistance to infection; but if we use the intravenous route, we should be testing the capacity of the tissues to resist the consequences

cities. These sand filters are constructed so that the water passes through 1 to 5 feet of sand supported upon graded layers of gravel (see Fig. 33). The rate of filtration must be accurately regulated and the efficiency of operation controlled by frequent bacterial tests of the effluent. Such filters are highly effective. Bacteria are removed, not to any great extent by mechanical straining out, but through a biological mechanism in which the activity of protozoa is an important feature. The passage of water through these filters is necessarily a relatively slow process and, in consequence, relatively large areas are required, which, for financial or other reasons, are no longer available in large American cities. Few of these filters have been constructed in recent years, and the use of rapid sand filters is becoming common.

The rapid sand filters, which may be used with turbid waters that would clog a slow sand filter, are frequently employed in conjunction with "coagulation," the addition of such substances as aluminum or ferric sulfate, which form flocculent precipitates (the hydroxides). The precipitate carries down most of the suspended matter and, of course, many bacteria, and is readily filtered out,

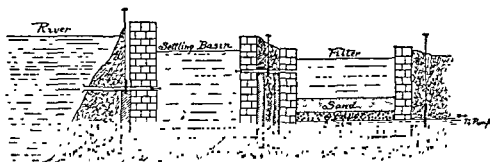


Fig. 33. Cross-section of filter plant (after Hazen).

yielding a clear effluent containing relatively few bacteria. Laboratory experiments on centrifugation of dilute suspensions of *Bact. coli* in the presence of kaolin or infusorial earth have indicated that 70 per cent of the viable cells are found in the sediment.¹⁸ Such filters must be cleaned from time to time, a process that is seldom required with slow sand filters. They make possible, however, the treatment of a large quantity of water on a relatively small filtering area.

Destruction of pathogenic bacteria in water supplies is most often brought about by treatment with germicidal chemicals. Of these, hypochlorite (calcium hypochlorite or bleaching powder) was at one time used widely in the treatment of municipal water supplies, and it still has its uses, although it has been largely superseded in large scale treatment by liquid chlorine, which is supplied in cylinders under pressure. Chlorine is added directly to the water through an automatic feeding device in accurately measured amounts which are determined by the character of the water. In general, the greater the amount of extraneous organic matter present, the greater must be the amount of chlorine added. The amount of chlorine taken up is termed the chlorine demand, and the point at which the residual chlorine or available chlorine becomes proportional to the added chlorine is called the "breakpoint" in the chlorine demand curve. The introduction of breakpoint chlorination usually

¹⁸ Schulhoff and Hueckelkian: Jour. Amer. Water Works Assn., 1936, 28.1963.

significantly lower epidemic mortality occurred in the groups whose diets included the dried milk. The better diet apparently reduced the risk both of manifest, and of latent infection.

Church (1939) recorded results substantially in agreement with these using *Salm. enteritidis* as the infecting agent; the dietetic effects were far more readily produced in young mice when the mothers were also subjected to the diet from a few weeks prior to mating. Indeed, the only changes found to exert a significant effect when instituted first in the weaned mouse occurred as the result of a 75 per cent. decrease in the total mineral constituents or an 80 per cent. decrease in calcium content.

Reference to the protocols of Webster and Pritchett (1924), Pritchett (1927), and Topley, Greenwood and Wilson (1931) shows that Watson's results were entirely compatible with the view that the milk powder in the earlier diets was the component that determined the increased resistance of the mice receiving it, though this possibility was not at that time envisaged. It would appear that the tissue changes on which increased resistance depends take some time to develop. The mode of action of the milk powder is obscure. The reproduction of Watson's mice was poor on the high oatmeal diet. Howie (1949) observed that reproduction of mice was better on low oatmeal than on high oatmeal diets, and suggested that Watson's milk powder diet had raised resistance by adjusting an imbalance in her poor diet. This may well be so, though it should be noted that a diet good for reproduction and growth of an animal is not necessarily the best for resistance to a given infection. Howie and Porter (1950), for example, among six diets tested, found no constant association between growth and reproduction on the one hand, and resistance to salmonella and the tubercle bacillus on the other.

Sengupta and Howie (1949) recorded that of two diets, one richer than the other in calcium, protein and vitamins promoted the greater resistance of mice to tuberculosis initiated by intravenous injection of the bacilli.

Schneider (1946b, 1948, 1949) examined a diet of whole wheat and whole dried milk, which had proved better than a "synthetic" diet in promoting resistance to oral *Salm. enteritidis* in mice. The effective factor proved to be a thermostable constituent of wheat, corn, yeast and grass. His experiments illustrate the importance of the genetic constitution of the mice used, and the state of the infecting organisms, which had to be nicely adjusted to reveal significant differences in resistance; the best results were obtained with a heterogeneous population of mice, and a mixture of virulent and avirulent forms of the salmonella.

In experiments on sheep, which were carried out in Scotland, animals were allowed to graze on different types of pasture, or fed on different diets in pens, and studies were made of the blood calcium, blood phosphorus, and (as an index of immunity *faute de mieux*) of certain of the natural agglutinating or lytic antibodies. The results, as one would expect, were by no means easy to interpret; but there appeared to be a definite correlation between ample pasturage, a high calcium and low phosphorus content of the blood, and general well-being, and there was a suggestion that these conditions were associated with active lytic properties of the serum, while the agglutinins in certain experiments appear to have varied inversely as the lysins (Orr *et al.* 1931). In later experiments (Mackie *et al.* 1932) a slight indication was obtained that the animals on the poor pasture were more susceptible than controls to the intradermal injection of the toxin of the lamb dysentery bacillus.

Proteins.—As regards the proteins, we may first note that experimental depletion of the serum proteins in dogs, by repeated bleedings and replacement of whole blood by erythrocytes alone, or experimental hypoproteinæmia and anæmia together, is associated with a high incidence of endocarditis, pneumonia and septicæmia (Robschey-Robbins, Miller and Whipple 1943). Hypoproteinæmia of rats and rabbits induced by low protein diets is associated with increased susceptibility to Type I pneumococcal infection, and a decreased formation of antibody, as evidenced by a poor response to specific immunization

results in material improvement in the sanitary quality of a water.¹⁹ When liquid chlorine is added to ordinary surface water, clear and not highly contaminated, in the proportion of about 0.5 to 1 part of "available chlorine" per million gallons, the ordinary intestinal bacteria are destroyed, including such pathogenic forms as the typhoid bacillus.²⁰ The tastes and odors in chlorinated waters may be due to an overdose of chlorine caused by inadequate control methods, or to the action of chlorine upon compounds present in the water, commonly as industrial wastes. Excess chlorine and tastes caused by chlorination can often be removed by dechlorination with SO_2 or treatment with KMnO_4 or activated carbon. The bactericidal activity of chlorine may be prolonged, particularly in waters containing considerable organic matter, by the simultaneous addition of liquid ammonia with the formation of chloramines.²¹ The introduction of chlorination of municipal water supplies has, in practically every instance, resulted in marked reductions in the incidence of enteric infection, typhoid fever in particular, in this country. The addition of chlorine or other bactericidal agents is not a "cure-all" however. When a water is sufficiently heavily contaminated, bacteria embedded in particles of organic matter are not killed even in the presence of available chlorine. It is generally agreed that a content of more than 50 coliform bacteria per milliliter indicates pollution too great for successful chlorination.

In the last few years chlorine dioxide (ClO_2) has been applied to the treatment of water. It has the advantages that it destroys algal tastes as well as the chlorophenol taste of certain chlorinated waters, and oxidizes organic matter more rapidly than chlorine, thus allowing the maintenance of a chlorine residual in the distribution system. In water solution this substance is decomposed by light to chloric and perchloric acids and oxygen, and is more bactericidal for coliform bacteria than chlorine.²²

It may be noted that frequently a combination of filtration and chlorination is desirable particularly with rapid sand filters. Not only does a preliminary filtration have esthetically desirable features in the case of turbid waters, but considerably less chlorine is required for treatment than would have been the case had not the greater part of the suspended material been removed.

The chemical treatment of water on a small scale does not always involve expense as a primary consideration and hence other methods are not infrequently used. The treatment of swimming pool water by ultraviolet irradiation adds no tastes or odor to the water and, although considerably more expensive than chlorination, can be used because the scale of operation is small. Ozone, which is strongly bactericidal, is also relatively expensive and is not commonly used in this country but is used to an appreciable extent in Europe.²³ Chlorina-

¹⁹ Cf. Griffin and Chamberlin: *Amer. Jour. Pub. Health*, 1945, 35:199.

²⁰ See, for example, the studies of Levine, Heller and Bender: *Jour. Amer. Water Works Assn.*, 1942, 34:1787.

²¹ The chlorine ammonia treatment is discussed in *Jour. Amer. Water Works Assn.*, 1941, 33:2079.

²² See Synana, McMahon and Vincent, *Water Works and Sewerage*, 1944, 91:423, *ibid.*, *Jour. Amer. Water Works Assn.*, 1945, 37:869, *ibid.*, *Amer. Jour. Pub. Health*, 1946, 36:1035.

²³ See the review on ozone treatment by Hann: *Jour. Amer. Water Works Assn.*, 1943, 35:585.

response, may obscure real differences in rates of synthesis, which might appear were the response to be tested at a lower level of efficiency. Thus, Howie, Barr and Glenn (1953) observed an increase in diphtheria antitoxin formation by ewes when a supplement was added to a grass diet, but only with the less efficient interval of 4 weeks between primary and secondary doses; when the interval between the stimuli was 9 weeks, the better response in both test and control animals masked this difference (see Balch 1950, above).

Minerals.—The possibility that variations in the intake of mineral salts might have an influence on resistance was explored by various experimental workers (Zilva 1919, Lange 1925, 1927*a, b*, Pritchett 1927, Hotta 1928, Kligler and Geiger 1928, Mayer and Sulzberger 1931, Sulzberger and Mayer 1931), but, excepting the results of Church (1939) noted above, the evidence is highly confusing.

Clark and his colleagues studied the effect of single mineral deficiencies in mice; variations in Ca, Mg and Cl had no effect on susceptibility to Theiler's encephalomyelitis virus; the incidence of paralysis, but not the death rate, was decreased by Na deficiency and by depletion of either K or P (Rasmussen *et al.* 1944, Lichstein *et al.* 1946). A rather different mineral effect was recorded by Hitchings, Falco and Sherwood (1949), who identified manganese as a dietary factor that in mice *enhanced* the infectivity of certain strains of pneumococci, apparently by supplying a growth factor to the organisms (see also p. 1387).

Apart altogether from their effect on the defence mechanisms of the tissues, there can be no question that variations in diet alter the local conditions in the gastro-intestinal tract. We know (see Rettger and Cheplin 1921, Dudgeon 1926) that the ingestion of certain foodstuffs produces characteristic changes in the gastro-intestinal flora; and Arnold (1926, 1927*a, b*, 1929) has stressed the importance of various food materials in deranging or maintaining the efficacy of the gastro-duodenal bactericidal mechanism, particularly in infants. He suggested that such results as those recorded by Webster and Pritchett (1924) and Pritchett (1927) might be due in part to effects of this kind.

We may note here an association between infection and hypersensitiveness to diet, which may prove to be of some importance in the study of resistance. Coca (1941) observed in man a syndrome that he ascribed to food allergy, and found that the incidence of the "common cold" was higher among allergic than among non-allergic human beings. Brown and others (1943) confirmed his observations.

Accessory Food Factors

The Fat-soluble Vitamins A and D.—In 1909 Knapp described in rats on a deficient diet the condition of xerophthalmia, which is characterized by a patchy thickening and drying of the epithelium of the eye, associated in its later stages with subacute bacterial infection of the underlying tissues. This condition later proved to be due to lack of the fat-soluble Vitamin A. It was found in other animals, such as the dog (Steenbock *et al.* 1921) and rabbit (Boock and Trevan 1922), and occurred in Danish children during the 1914-18 war (Bloch 1921, 1923). The bacterial infections are not limited to the eye. Bloch noted that the xerophthalmic children were unable to withstand infections of any sort, and died quickly of serious intercurrent fever. The most important killing disease was broncho-pneumonia. Blegvad (1923) recorded the following incidence of serious complicating infections among the cases of xerophthalmia—broncho-pneumonia 63, bronchitis 45, pyuria 42, otitis media 30. These clinical observations are in *entire* accord with the results obtained in dietary experiments on animals.

The liability of rats fed on a diet deficient in Vitamin A to develop intercurrent infections, such as glandular abscesses, suppurative arthritis, suppurative

tion is, of course, always available. It is carried out by the addition of hypochlorite when the installation of liquid chlorine apparatus is not desirable, as, for example, in the treatment of a contaminated cistern.

At times the treatment of water becomes an individual matter, as in the case of an army in the field or when a public supply is known to be impure. In the first instance the water may be treated by the addition of hypochlorite in the form of bleaching powder or a solution of sodium hypochlorite, or by the addition of a small amount of iodine, one part of Lugol's iodine solution to 1000 parts of water. In the home, filters, such as the Berkefeld or Chamberland bougies, may be used, but filtration is slow and care in operation and frequent cleaning are necessary. The simplest and best method of water treatment for the family or individual, however, is simple boiling. Boiling for five minutes is quite sufficient to destroy with certainty the typhoid bacillus and allied forms as well as the cholera vibrio. When water-borne disease is prevalent, or when a water supply is notoriously impure or exposed to chance of infection, boiling is the only wholly safe procedure.

SEWAGE

Sewage is best regarded as the used water supply of a community and as such is a dilute solution of fecal matter and other wastes. From the hygienic point of view it is an important vehicle in the transmission of enteric infection, hence the manner of its disposal is of considerable significance. The mechanisms of sewage disposal have as their objects first, the ridding of a community of an ever-present volume of waste and second, disposal in such a manner that it is not dangerous to other communities.

The complex organic compounds present in sewage undergo the same processes of decomposition that are involved in the breakdown of dead organic matter in nature and are a part of the so-called cycles of elements such as nitrogen, phosphorus and the like. Any type of sewage treatment, then, is nothing more than a mechanism for bringing about or accelerating these transformations. The organic compounds are first broken down to amino acids, monosaccharides and the like, which are eventually oxidized completely to carbon dioxide and water in the case of carbon and hydrogen, and to nitrite and nitrate in the case of nitrogen. Bacteria are the active agents in this decomposition and oxidation, the mechanisms of which have been discussed earlier (Chapter 4). Although essentially simple in principle, sewage treatment is in practice a complex problem which cannot be considered at length here.²⁴

In general, however, sewage disposal falls into one of three categories: (a) dilution, (b) partial treatment and (c) complete treatment. In the first instance the sewage is simply dumped into some body of water where it will not annoy its originators. Here the breakdown and oxidation of the constituents of the sewage occur in nature, and, if sufficient time elapses, no trace remains beyond an increase in nitrate which, in turn, serves as food material for phytoplankton. When this transformation takes place in a flowing stream, the phenomenon is known as the *self-purification of streams*. The essential element, however, is not the fact that the water is in movement but that sufficient time

²⁴ See Imhoff and Fair. *Sewage Treatment*. John Wiley & Sons, New York 1940.

rats suffered from spontaneously acquired infection, which may itself have affected the phagocytic index. The significance of lysozyme in defence against infection is not clear (see Chapter 45), but it may be noted that in deficient rabbits Sullivan and Manville (1937) found a retention of lysozyme in the intestinal tissues, and a diminution in the mucous secretions.

Observations have been recorded suggesting that the deprivation of Vitamin A renders animals more susceptible to the injection of bacterial toxins, as well as to the invasive action of living bacterial cells (Werkman, Baldwin and Nelson 1924, Schubert 1928). Torrance (1936), however, in a careful study of the Vitamin A content of the liver in guinea-pigs, found no association between vitamin level and susceptibility to diphtheria or tetanus toxin (see also Chalier and Jeune 1938).

It is possible that the whole effect of Vitamin A deficiency is indirect. Kligler, Guggenheim and Henig (1945) found, in accordance with a number of other observers, that resistance of rats and mice to infection—in their case with *Salm. typhi-murium*—was not significantly lowered by a partial Vitamin A deficiency, but only by a complete avitaminosis. Avitaminosis was, however, invariably associated with declining weight in the experimental animals. In other words, the decline in resistance appeared to be due to starvation, a conjecture supported by the fact that rats whose food intake was restricted to the same amount as that eaten by avitaminotic rats but whose Vitamin A intake was normal, were as susceptible as the avitaminotic animals to the salmonella infection.

We have been concerned so far with Vitamin A deficiency, which appears to be moderately disadvantageous to the animal. There is little evidence, either experimental or from the field, to determine whether excess of the vitamin is beneficial.

In mice orally infected with *Salm. typhi-murium*, Pritchett (1927) observed that withdrawal of butter-fat, as a source of Vitamin A, from the McCollum diet (p. 1367) containing wheat flour, casein, milk powder and salts decreased resistance; and its addition to a diet of bread soaked in milk, with supplements of oatmeal and buckwheat, increased the resistance.

But with contact infections during an experimental *Salm. typhi-murium* epidemic in mice, Topley, Greenwood and Wilson (1931) found that the addition of excess Vitamin A in the form of butter-fat, or a vitamin concentrate, or as provitamin A in carrots, to a basal diet of wheat flour, casein, butter and a salt mixture, slightly increased susceptibility, whereas these supplements did not affect the mortality of intraperitoneally infected mice.

These experiments have a bearing on the effect of an excess of Vitamin A over the amount required to maintain growth and well-being; but it would be unwise to lay much stress on their significance, since the diets employed varied in many ways besides their differences in Vitamin A content. We may conclude that no adequate experimental evidence has yet been produced to show that an excess of Vitamin A over the level required to prevent all overt signs of avitaminosis induces an increased resistance to bacterial infection.

Little evidence is available with regard to the effect of increasing the amount of Vitamin A supplied to human subjects. Antibody formation has not received much attention. Scaglione (1938) concluded from a study of rabbits and young children that Vitamin A improved the agglutinin response to T.A.B. vaccines; and Schmeckebier (1943) reported an increase of diphtheria antitoxin in the blood of women given Vitamins A and D. As regards resistance to infection itself, the bacterial complications of child-birth afford opportunities for such a trial, and are of particular interest because of the obvious possibility that pregnancy may make a large call on the vitamin reserves. Mellanby, Green and their colleagues (Green *et al.* 1931) recorded a trial in which 275 women received a Vitamin-A concentrate

elapses for the breakdown and oxidations to proceed to completion. Soil polluted with sewage similarly "purifies itself."

With increasing population densities the disposal of sewage by dilution becomes unsatisfactory because a body of water is not infrequently a water supply to a neighboring community. Some form of treatment, then, becomes obligatory, in other words, the decomposition is made to take place in whole or in part in the various tanks and other devices making up a sewage treatment plant rather than allowing it to occur in natural bodies of water. In practice, treatment takes the form of preparatory processes followed by a period of *anaerobic digestion*, then a period of *aerobic oxidation*. Treatment may be complete even through nitrification, or the partially treated sewage may be disposed of by dilution.

When the disposal of fecal material is an individual or family problem the mechanics involved are somewhat different, but the processes of decomposition and oxidation are essentially the same whether a privy, cesspool or septic tank is used.

It will be noted that, although sewage treatment is basically a bacteriological process, the pathogenic bacteria are not involved; their presence is not taken into consideration, nor is there any effort to destroy them except in the rare instances in which the effluent from a complete treatment plant is chlorinated. In one sense, then, sewage treatment is not directed toward the control of water-borne disease. The fact that it is treated, however, is of considerable significance in this connection. When sewage is disposed of by dilution, the typhoid bacillus and related microorganisms do not multiply; rather, a decline in numbers sets in immediately, and these bacteria do not survive as long in water as in soil, probably not more than a few days or a week. It is probable that they tend to disappear equally rapidly during sewage treatment, although

economic and esthetic aspects of sewage treatment are, of course, another matter.

Vitamin C (ascorbic acid). In the earlier records there are suggestions that B-avitaminosis is associated with a minor increase in susceptibility to infection, but the evidence is of very doubtful significance (see Werkman 1923a, b, Werkman, Baldwin and Nelson 1924, Lassen 1929, 1931, 1932, Robertson 1934).

The effect of a prolonged suboptimal intake of the vitamin B complex was studied in rats by Drummond and others (1938). The deficient rats had feebler reproductive powers, were shorter-lived, and *post mortem* showed a significantly higher incidence of gastro-intestinal lesions than those in a control group on a full diet. Nevertheless, they suffered from no epidemics, and the incidence of bronchiectasis, the only infective disease to which the rats were prone, and of sporadic pneumonia and abscesses, was the same in deficient and control groups. Rose and Rose (1936) compared the course of artificial *Staph. aureus* bacteraemia in 8 normal and 8 B₁-deficient dogs. The deficient dogs lost weight more rapidly, and suffered a more prolonged bacteraemia, but there was no significant difference in the recovery rates in the two groups. They found no depression of resistance to *Cl. welchii* toxin in deficient dogs (Rose, Rose and Kolmer 1936). Janota and Dack (1939) record that the anaemia, leucopenia, gingivitis, oedema and diarrhoea which follow B₂ deficiency in monkeys is associated with the spontaneous appearance of *Sh. flexneri* in the stools. Rats fed on B-deficient (Lamb 1935) or thiamin-deficient diets (Badger, Masunaga and Wolf 1940) exhibit a decreased resistance to rat leprosy. Wooley and Sebrell (1942) maintained mice on thiamin- and riboflavin-deficient diets for 2-3 weeks, and found a diminished resistance to intranasal infection with Type I pneumococci. Day and McClung (1945) observed a slight diminution of resistance to intraperitoneal infection with pneumococci in pantothenate-deficient mice, but not in similarly treated rats. Robinson and Siegel (1944), using comparatively small numbers of animals, studied the effect of restricted B vitamin intake on rats injected intratracheally with a mixture of mucin and Type I pneumococci. Compared with rats on a defined "synthetic" diet containing adequate amounts of known B vitamins, neither riboflavin- nor pantothenic acid-deficient rats were less resistant; thiamin deficiency, and to a less significant degree, pyridoxine deficiency, lowered the resistance. Rats on a "normal" stock diet, it should be noted, were as susceptible as thiamin-deficient rats.

Guggenheim and Buechler (1946) found in mice a decreased resistance to oral infections by *Salmonella typhi-murium* that was attributable to thiamin deficiency. In their thiamin-deficient rats, however, the decrease they observed was due to the accompanying inanition due to poor caloric intake. Inanition, rather than vitamin deficiency, appeared also to be the cause of increased susceptibility to relapsing fever spirochaetes in thiamin-deficient rats (Guggenheim and Buechler 1946). Folic acid deficiency in monkeys, carried to the point where the animals were leucopenic, was reported greatly to decrease resistance to infection with influenza virus and with streptococci initiated by the nasal route (Saslaw *et al.* 1946).

The severity of rickettsial infections of the rat and the guinea-pig are increased on a starvation diet (Zinsser *et al.* 1931.) Vitamin-A deficiency does not depress the resistance of the rat to murine typhus, but riboflavin deficiency does so strikingly; and repair of the deficiency, even in the late stages of a fatal infection, is curative (Pinkerton and Bessey 1939).

Number of B vitamin deficiencies in rats, Fitzpatrick (1948) found that only

Amongst the B vitamins, only B₁ and B₂ deficiencies were found to have a marked effect on resistance to infection. Kuczynski (1937) increased the survival rate of normal mice injected with yellow fever virus from about 20 per cent. to about 75 per cent. by increasing the dietary thiamin. It is doubtful whether these effects are specific. For example, Sabin (1941) has shown that though thiamin or B-complex deficiency delays the normal acquisition by mice of

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receiving adequate Vitamin C and of those on a vitamin-deficient diet. Vogl (1937) reported that Vitamin C prevented the development of post-operative pneumonia, and that it hastened the convalescence of pneumonic patients (see also Scnewald 1938, Szirmai 1940). Beneficial results have been claimed also in the treatment of whooping cough (Ormerod and Unkauf 1937), though Gairdner (1938) in a carefully controlled test found no difference between untreated and vitamin-treated children. Bamberger and Zell (1936), Dieckhoff and Schüler (1938) and Szirmai (1940) also claimed beneficial results in diphtheria. In most cases, however, the number of uncontrolled factors in these tests was large, and conclusions as to the specific action of the vitamin must be drawn with circumspection.

Glazebrook and Thomson (1942) provided one of the most clear-cut indications of a specific Vitamin C effect. They studied two groups of boys, one deficient in, the other saturated with, Vitamin C. They could find no difference in the incidence of common colds or tonsillitis in the two groups, but noted that deficient patients with tonsillitis stayed in hospital 16.7 days on the average, as against 10.0 days for the saturated patients. During the period of observation there were 17 cases of pneumonia and 16 cases of acute rheumatic fever among 1,100 deficient boys, and none among 335 saturated. The association of infection with low vitamin levels in animals or man is not in itself good evidence that the one is a direct result of the other, for in the infective state, especially when it is accompanied by fever, there is a decrease of the vitamin in the tissues; indeed, infection may precipitate scurvy in persons suffering from hypovitaminosis (see Abbasy *et al.* 1937*a, b*, Harris *et al.* 1937, Fox 1943). The point is well illustrated in the attempts of Rinehart and his colleagues to reproduce the lesions of rheumatic fever in guinea-pigs. They found (1934) that infection of scorbutic guinea-pigs with hemolytic streptococci resulted in certain cardiac lesions which did not appear in scorbutic animals, or in normal animals infected with the streptococci. Moreover, in patients with rheumatic fever, the vitamin levels were unusually low (1936). McBroom and his colleagues (1937), on the other hand, observed the lesions in uninfected scorbutic guinea-pigs, provided that the scurvy was severe enough, and suggested that Rinehart's lesions were due to severe scurvy, precipitated in a non-specific manner by the superadded infection.

As regards spontaneous infections in guinea-pigs suffering from experimental scurvy, Hamburger and Goldschmidt (1922-23), Grant (1926, 1930) and Schmidt-Weyland and Koltzsch (1927) state that they are very common. On the other hand, Holst and Frölich

as relevant evidence, though we clearly cannot assume that C-avitaminosis is a determining factor.

As regards the resistance of scorbutic guinea-pigs to experimental infection, Findlay (1923), Werkman, Nelson and Fulmer (1924), Grant (1926) and Schmidt-Weyland and Koltzsch (1927) recorded a slight increase in susceptibility. Findlay ascribed this to the degenerative changes and feeble leucoblastic reaction seen in the bone-marrow of guinea-pigs suffering from chronic scurvy. Werkman, Nelson and Fulmer stressed the association between lowered resistance and lowered body temperature.

The observations of Zinsser and Castaneda (1931) on the increased susceptibility of guinea-pigs and rats on a vitamin-deficient diet to experimental typhus infection may be referred to here. It is probable that the guinea-pigs were suffering from some degree of C-avitaminosis; but the fact that rats, which are generally regarded as being relatively insensitive to the deprivation of this vitamin, showed the same decrease in resistance makes it difficult to assess the significance of these results.

McCullough (1938) recorded an increased susceptibility to infection by *F. necrophorus* in deficient guinea-pigs, which only became evident when the scurvy was severe; the administration of Vitamin C was followed by prompt recovery. In man, major deficiency is associated with a retardation of fibroblastic activity and of wound healing (see Hunt

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pantothenate and pyridoxine, but not riboflavin (Axelrod *et al.* 1947); thiamin and biotin (Carter and Axelrod 1948); pyridoxine (Agnew and Cook 1949); pyridoxine, but not pantothenate or riboflavin (Stoerk *et al.* 1947); pantothenate (Ludovici *et al.* 1949); folic acid and tryptophan and nicotinic acid but not cyanocobalamin (Ludovici and Axelrod 1951); pyridoxine, pantothenate, thiamin, riboflavin, folic acid, but not nicotinic acid (Wertman *et al.* 1951, 1952); pantothenate (Giunchi *et al.* 1953). The different experiments were not of course all strictly comparable; but pyridoxine deficiency is consistently reported as impairing antibody production. Folic acid and biotin deficiencies and, with one exception, pantothenate deficiency are also definitely depressant. Riboflavin and thiamin deficiency have less well established effects.

In the mouse, Ruchman (1946) found that lack of pyridoxine, but not of thiamin or riboflavin, was depressant; and in the chick, Little, Oleson and Roesch (1950) that folic acid, but not cyanocobalamin, decreased immunizability.

The evidence with regard to the immunological consequences either of excess or deficiency of Vitamin C is more confusing.

Lesné and Dreyfus (1911) and Zolog (1924) contended that in guinea-pigs deficiency was associated with diminished sensitivity to anaphylactic shock. Cohen (1938) was unable to confirm these findings (see also Sartori 1925, v. Nickerk 1937). Several observers report a prophylactic effect of excess Vitamin C in guinea-pigs, both in the whole animal, and in isolated sensitized tissues (Lemke 1936, Solomonica 1936, Ungar, Parrot and Levillain 1937). Hochwald and Schwartz (1937), Raffel and Madison (1938) and Storck (1939) were unable to confirm these findings, and Dragstedt (Dragstedt *et al.* 1938) failed to find any effect of Vitamin C on hypersensitive dogs. Similarly with antibody formation; Juszat (1936b) and Madison and Manwaring (1937) recorded enhancement of precipitin response in rabbits, and Raffel and Madison (1938) in guinea-pigs given excess Vitamin C. Hochwald and Schwartz (1937) found no enhancement in guinea-pigs immunized with horse serum, and Madison, Fish and Frick (1938) no effect on the response to bacterial antigens. Long (1950), however, recorded a 30-fold depression of diphtheria antitoxin response during the immunization of guinea-pigs on a diet deficient in ascorbic acid, compared with control animals given either an ascorbic acid or a cabbage supplement. Cabbage supplements were also found by Hartley (1948) to improve antitoxin response in the guinea-pig in proportion to the amount given. The active factor in the cabbage is unknown. (For a discussion of the relation between Vitamin C and serum complement, see Chapter 7.)

If we attempt to assess the significance of the data at present available with regard to the influence of variations in vitamin intake on resistance to infection, we shall conclude (1) that an excess of any vitamin over the normal dietary requirements has no consistently demonstrable effect on resistance; and (2) that certain gross vitamin deficiencies are associated with a significant decrease in resistance. In some cases the effect is indirect, in that the deficiency has impaired the intake of protein and calories, so that general inanition, not a particular deficiency, is the immediate cause of lowered resistance. When inanition is as far as possible excluded, it appears that deficiency of Vitamin A, but not of Vitamin D, decreases resistance to some extent. As regards the water-soluble vitamins, resistance to bacterial and rickettsial infections appears to be lower in riboflavin, pantothenate and folic acid deficiencies. Resistance to certain neurotropic viruses is increased by thiamin and pantothenate deficiencies. Lack of other B vitamins and of ascorbic acid may also decrease resistance, but the evidence is more divergent. The resistance of the guinea-pig is most readily affected by ascorbic acid deficiency, as might be expected of an animal that does not itself synthesize this vitamin.

It is clear that we cannot generalize for infections as a whole; each vitamin

THE BACTERIOLOGY OF MILK AND FOOD

MILK¹

As a vector of infectious disease, milk differs from water in that it is an excellent medium for the growth of many pathogenic bacteria, and from other foods in that it is the only food of animal origin that is consumed in large part in the raw state. Since large quantities of milk are consumed—it is estimated that about 16 per cent of the average dietary in the United States consists of milk and milk products—the importance of this substance in the transmission of disease is evident. The diseases transmitted by milk are: first, diseases of cattle transmissible to man and including bovine tuberculosis, undulant fever, foot-and-mouth disease and streptococcal infections from infected udders; and, second, diseases of man in which the milk serves as the link between man and man, such as typhoid fever, septic sore throat, scarlet fever, diphtheria and, rarely, certain other diseases, such as poliomyelitis.

Sources of Bacteria in Milk. Unlike water, milk has no native bacterial flora, and it is probable that milk as secreted into the udder of a healthy cow is sterile. The milk in the udder is, however, rarely if ever bacteriologically sterile, for the microorganisms invade the udder via the milk ducts of the teats and the first portion of the milk drawn (fore-milk) always contains more bacteria than the last (strippings). There is, furthermore, no bacterial flora that is characteristic of milk; the presence of microorganisms is always a consequence of contamination and the types of bacteria found are determined by the source of contamination. From this point of view the bacteria of milk fall into two groups: first, those which are present in the tissues of an infected cow and find their way into the udder, and, second, those which enter the milk, usually after it is drawn, from sources external to the animal.

Bacteria from Infected Cattle. Of these microorganisms perhaps the most important is the *tubercle bacillus*. These bacteria get into the milk directly as a consequence of tuberculosis of the udder, which occurs in 1 to 2 per cent of infected cows, and indirectly by contamination with cow manure when the infectious sputum is swallowed and discharged with the feces. In either case the milk is infective for man, the bovine variety of the tubercle bacillus giving rise, as a rule, to bone and joint rather than pulmonary tuberculosis (p. 631). Milk may, of course, be infected with human tubercle bacilli from infected persons. A high proportion of the former type of infection occurs when the transmission of this disease is allowed to occur. The disease may be controlled at its source, i.e., by the elimination of infected cattle from dairy herds, a practice which is practically universal in the United States.

¹ Hammer: *Dairy Bacteriology*. 3rd ed. John Wiley and Sons, New York. 1948.

Three to six days after an intracerebral dose of virus the animals were exercised to the point of fatigue by swimming in water at 39° C.; the incidence of the disease and the severity of paralysis were higher in these than in control animals, but immersion in the water without exercise had a similar effect. That the chilling incidental to immersion may have been operative was evident from the result of cooling the animals some 11–12° C. by immersion for 30 min. at 17° C.; the incidence of the disease was thereby doubled. More direct effects of fatigue are demonstrable; for example, Rosenbaum and Harford (1953) raised the incidence of disease and the death rate in mice that were made to exercise for one day preceding, and on each day after an intracerebral injection of poliomyelitis virus.

The Effect of Variations in Temperature, Humidity and Other Physical Conditions of the Environment

The evidence available under this head is concerned mainly with the effect of these environmental factors on the first-line defence mechanisms of the kind considered in Chapter 45. Arnold and his colleagues (Arnold 1927*b*, 1928, 1929, Arnold and Brody 1927) studied in considerable detail the effect of high temperature, especially when associated with high humidity, on the gastro-duodenal bactericidal mechanism of the dog. They found that such conditions were associated with a decrease in gastric secretion, a consequent fall in the acidity of the gastric and duodenal contents, and an increased passage of bacteria from the stomach or duodenum to the caecum.

There are in the literature many other records of experiments on the effect of temperature and humidity on resistance to infection (see McDowell 1923, Khigler and Oltzki 1931, Robertson and Weld 1932, Nishikawa 1935), but the number of animals in the comparable groups are so small, and the effects of these two factors seem so difficult to disentangle, that the significance of many of the results reported cannot be assessed.

Sulkin (1945) emphasizes the contradictory results obtained in many experiments, even with the same species of animal and the same species of parasite. The general indication, however, with a number of types of infection, is that exposure to low temperatures, if it has any effect, decreases the animal's resistance. But the effects are often peculiar to the conditions of the experiment. Chilling may directly affect the multiplication of the parasite, or the defence mechanisms; but it may also inhibit the development of symptoms of the disease without materially affecting the degree of parasitism. We may note a few examples.

In the chick embryo, Beveridge (1944) reported that infections with influenza virus by the chorio-allantoic route were less effective in eggs at 39° C. than at 37° C., but the change in temperature did not alter infectivity via the yolk sac or amnion. Sim (1949) recorded a lowered resistance to and greater generalization of infection by vaccinia virus at subnormal incubation temperatures, the effects of which could to some extent be reversed by restoring the eggs to optimal temperatures, and Khigman, Crane and Norris (1951*a*) reported that cryptococcal infections of the embryo disappeared more rapidly at 40° C. than at 37° C.

With influenza virus infection of mice induced intranasally, Sulkin (1945) observed no difference in the mortality of animals kept at 13° C. and 25° C., though the pulmonary lesions were less at the higher temperature. Holtman (1946*a*) acclimatized mice to temperatures of 13° C., 22° C. and 32° C., both before and after infection with poliomyelitis virus, and found a direct relation between the survival time and environmental temperature, as though the higher metabolic rate in the warmer animals had facilitated infection with the virus. Mills and Schmidt (1942) found that mice acclimatized at about 20° C. survived a lethal dose of pneumococci for twice as long as mice acclimatized to 32° C.; though all mice had the same body temperature. Nevertheless, according to Junge and Rosenthal

A bovine variety of *Brucella melitensis*, the causative agent of undulant fever (Chapter 23), infects cattle, producing the disease contagious abortion (the bacterium is sometimes designated *Brucella abortus*). This microorganism is excreted in the milk and infects man, producing a mild type of undulant fever. The caprine variety of this bacterium which is present in the milk of infected goats produces a much more severe disease in man. Undulant fever, like tuberculosis, may be controlled by control of the disease in the animal reservoir of infection.

The virus of foot-and-mouth disease, a virus disease of cattle, is excreted in the milk and is thus transmitted to man. The disease in man is mild, however, and not of great public health importance. More important are the streptococcus infections of the udder, designated garget or mastitis. The *Hotis test* is commonly used for the detection of mastitis; it consists of incubating fresh milk in the presence of 0.025 per cent bromocresol purple for twenty-four hours at 37° C. A positive reaction, the formation of yellow flakes on the side of the test tube, is dependent upon the presence of the streptococci and of agglutinins in the milk, the growing bacteria are clumped by the antibody and the acid reaction results from the fermentation of lactose. The indeterminacy of streptococcus species, coupled with the fact that the same type of streptococcus may produce more than one disease (p. 367), makes it difficult to evaluate the significance of streptococcus mastitis to milk-borne disease. Milk-borne epidemics of septic sore throat, however, are not infrequently associated with acute udder inflammation in the dairy herd and the massive and continuous infection occurring in some of the outbreaks indicates that the udders of the cattle were infected. It has been suggested that the streptococci of scarlet fever may proliferate, with or without symptoms, in the udder, and epidemics of human streptococcal infection, septic sore throat and scarlet fever, may occur,² though it is probable that in most instances milk-borne scarlet fever is a consequence of direct human contamination. It may be noted that the udder may be infected occasionally with diphtheria bacilli, which produce small external ulcers, but this is an uncommon occurrence.

Bacteria from External Sources. When milk is collected under ordinary conditions, the udder bacteria form but an insignificant fraction of the total number of microorganisms in the milk. The skin of the cow, the hands of the milker, the vessels used for collection, and the dust of the cow barn all contribute their quota to the number of bacteria found immediately after milking. If milk is obtained with aseptic precautions, it contains only a few hundred (200 to 400) bacteria per milliliter; collected with somewhat less care, it may contain a few thousand (2000 to 6000); with careless manipulation, even freshly drawn milk may be highly contaminated (30,000 to 100,000 per milliliter). If milk is kept at 0° C. (32° F.), it shows a decrease in the bacterial content during the first few hours, but at higher temperatures the rate of multiplication is high and, when richly seeded at the outset, enormous numbers of bacteria result.

The non-pathogenic bacteria that are present in milk are often differentiated on a physiological basis into the following groups:

- (1) the acid forming bacteria,

² Evans. Jour. Inf. Dis., 1946, 78 18.

non-infective doses of pneumococci would infect the lungs of rats when introduced together with mucin; and that though chilling by itself did not lower the resistance of rats to intraperitoneal pneumococci, it greatly increased the incidence of pneumonia in rats inoculated intranasally with mixtures of pneumococci and mucin. They concluded that chilling, by increasing the aspiration of mucous secretions from the upper respiratory tract, might favour the occurrence of pneumonia.

Though they are not concerned with the direct effect of cold, we may note the experiments of Locke (1937) who observed that the more rapidly rabbits recovered their body temperature after artificial chilling, the greater was their resistance to subsequent intravenous and intradermal infection with pneumococci. Here the chilling was employed as an independent test of the physiological response of the animals to an adverse environment. Duerschner, Muschenheim and their colleagues (Hardy *et al.* 1943, Duerschner *et al.* 1943, Muschenheim *et al.* 1943) reported upon the effect of severe chilling in experimental tuberculosis and in pneumococcal infections. Rabbits and guinea-pigs were held under anaesthesia for 6 to 24 hours at 4–8° C. twice weekly for several weeks. The treatment had no effect on the development of tuberculosis in guinea-pigs, though there was a long delay in the appearance of skin sensitivity to tuberculin. The response of chilled and normal rabbits to an intradermal lethal dose of virulent pneumococci was similar, except that the local lesions were smaller in the chilled animals. When less virulent pneumococci were injected, chilling resulted in inhibition of the local lesion, and a fatal bacteraemia. The rapid invasion of the blood stream did not appear to be solely due to the absence of a local response, for in other experiments chilling was found to reduce the minimal lethal dose even of intravenously injected pneumococci (see also Bruneau and Heinbecker 1944).

Ellingson and Clark (1942) reported a series of experiments to test the response of various defence mechanisms to temperatures greater than normal. In rabbits the artificial induction of a severe fever (41.5° C.) slightly reduced the antibody response during immunization with various antigens. It also produced a slight acceleration of the rate of disappearance, and presumably, of destruction of antibodies in the blood stream. Phagocytosis of *Staph. aureus* by human and guinea-pig leucocytes was only slightly depressed at 42° C.; and in rabbits injected intradermally with lethal doses of virulent pneumococci, the maintenance of artificial fever until the infection itself reached the febrile stage made no significant alteration in death rate, survival time, or degree of bacteraemia.

The temperature relations of *in vitro* phagocytosis were also investigated by Clark and his colleagues. With guinea-pig leucocytes, for example, both opsonization and phagocytosis of staphylococci rose to a maximum with rise in temperature from below 37° C. to 42° C., and declined sharply with further rise. But in changing from 37° C. to 32° C. the efficiency of phagocytosis did not decrease by more than 10–15 per cent. (see Zarafonetis, Harmon and Clark 1947). As regards the clearing mechanism of the reticulo-endothelial system, the clearance of carbon particles from the blood of rats is reported to be impaired in rats kept at a body temperature of 22° C. to 26° C. (Halpern *et al.* 1951).

Among other physical agencies mention may be made of the possible effect of ultraviolet irradiation of the body surface, since this procedure has been advocated as a means of increasing resistance to infection. We have already noted (p. 1349) that Colebrook, Eidenow and Hill (1924) demonstrated a temporary increase in the bactericidal power of the blood as the result of exposure to ultraviolet rays, and a similar effect after exposure to dark heat, or to the blistering of the skin with a mustard plaster. The general experience of the beneficial effect of sunlight as enjoyed under optimal natural conditions, combined with such observations as the above, made it natural to inquire whether any isolated constituent of the solar radiation, and particularly the rays of short wave-length, would produce a significant increase in resistance to infection. Some favourable views have been recorded (Maughan and Smiley 1928, 1929, Hill and Laurie 1931); but the balance

- (2) the alkali-forming bacteria,
- (3) the proteolytic bacteria and
- (4) the inert bacteria.

The first group includes the fermentative bacteria, and the most common type of fermentation is the lactic acid fermentation, the process by which milk usually sours under natural conditions. A variety of bacteria may be responsible, among them such familiar forms as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacterium coli*. A few species, however, are commonly active in the natural souring of milk, and these may be divided into two groups. One of these is comprised of the capsulated gas-forming bacilli of the *Bacterium (lactis) aerogenes* type, which are closely related to *Bact. coli*, differing principally in their possession of capsules, lack of motility, and ability to produce gas from potato starch. The second type, a streptococcus, *Streptococcus lacticus* (or *lactis*), is abundant in naturally soured milk, particularly when the acidity is high.

Lactic acid milks, regarded by some as having therapeutic value in the treatment of certain intestinal disorders, have been prepared by the inoculation of *Lactobacillus* species such as *acidophilus* and *bulgaricus*. The lactobacilli, however, are commonly found in the fermentation of ensilage and are present in small numbers in the human mouth and intestinal tract and are not ordinarily responsible for the natural souring of milk.

Although lactic acid is commonly the predominating acid in the fermentation of milk, the formation of butyric acid is observed occasionally. This is generally a consequence of the presence of the anaerobic butyric acid bacteria, but may be brought about through the agency of certain aerobes closely allied to *Bacillus subtilis*.

The spontaneous alcoholic fermentation of milk is less usual under natural conditions than either the lactic or the butyric, and the preparation of certain alcoholic beverages is dependent upon the artificial production of this form of milk fermentation. Koumiss, a drink prepared by Tartars by the alcoholic fermentation of mare's milk, and kefir, an effervescent sour milk prepared by inhabitants of the Caucasus from the milk of cows, goats and sheep, are both prepared by the inoculation of fresh milk with old koumiss in the first instance and with "kefir granules" in the second. The bacteriology of the koumiss fermentation is not well known, but in the case of kefir both a bacterium and a yeast appear to be involved. Some species of yeast, it may be noted, are able to effect the alcoholic fermentation of milk in pure culture.

The *alkali-forming bacteria* are those organisms which do not ferment lactose but, presumably, act upon the nitrogenous substances present with the liberation of ammonia. When typhoid and paratyphoid bacilli, for example, are cultivated in litmus milk no effect is apparent beyond a slowly increasing alkalinity. Certain other bacteria, such as some of the aerobic spore-forming types, also produce lipase and decompose the fats present, converting the milk to a yellow, transparent fluid.

The *proteolytic bacteria* also produce an alkaline reaction and, in addition, hydrolysis of the milk proteins. Two enzymes, or groups of enzymes, are responsible, one, a rennin-like enzyme, precipitates the protein with the formation of a soft curd, and a second, casease, brings about hydrolysis of the protein

to escape from the view that they must have been due to synchronous transient fluctuations in the resistance of the mice, but these fluctuations showed no definite seasonal distribution.

Even if the occurrence of seasonal variations in resistance were clearly established, it would be an exceedingly difficult task to disentangle the various factors involved; and without such disentanglement there could be little hope of intelligent interference. The vitamin content of foodstuffs, and many other dietetic variables, are subject to seasonal influences; the condition of the nasal mucosa and the efficiency of the gastro-duodenal bactericidal mechanism are, as we have seen, affected by changes in temperature and in humidity; and there is little doubt that many other types of physiological response, of which as yet we know little or nothing from the immunological viewpoint, may be subject to influences of a similar kind. Until our knowledge of the relative significance of these factors, working in isolation, is much more accurate and detailed than it is at present we can hardly hope to construct a useful working chart of the total effect of the environmental changes associated with the changing seasons. (See Aycock *et al.* 1945).

The Effect of Certain Chemical Substances in Enhancing Bacterial Infection

We may include in this chapter a brief survey of a series of observations that have, during recent years, focussed attention on certain chemical substances that play a significant part in the local pathogenesis of infective lesions. Some are important because they play a part in the infective hazards of the human and animal environment, others because they throw light on the mechanisms of immunity.

Calcium salts.—We may begin with the observations of Bullock (Gye) and Cramer (1919) on the effect of calcium salts in certain anaerobic infections, and the later studies of Fildes (Fildes 1927, 1929*a, b*, Knight and Fildes 1930, Campbell and Fildes 1931) Bullock and Cramer found that ionizable salts of calcium, inoculated together with the washed spores of *Cl. welchii*, of *Cl. tetani* or of certain other anaerobes, led to the development of the corresponding infections in their most typical and fatal forms, whereas the washed spores alone showed no tendency to develop in the tissues after inoculation. The chlorides of sodium, potassium, ammonium, strontium or manganese had no such effect. They were unable to determine the exact mechanism by which this effect, to which they gave the name *kataphylaxis*, was brought about; though they were able to show that it was not due to an absence of leucocytes from the lesions, or to a lack of phagocytic activity on the part of the cells that collected at the site of inoculation. Fildes (1927), attacking the particular problem of experimental tetanus in the guinea-pig, studied the vegetation of spores in the normal testicle, after ligation of the blood vessels. He found that the injection of tetanus spores into the ligated testicle was followed by a rapidly fatal toxæmia, indicating rapid vegetation in the tissues. Comparison of sections from ligated and unligated testicles, at various intervals after the injection of the spores, showed that active vegetation had occurred in the ligated testicle at a period when emigration of leucocytes was at its earliest stage in the unligated

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 tentatively rejected this view. Later (Fildes 1930) he . . .
 oxidation-reduction potential of the tissue fluids under different conditions using suitable indicator dyes. He found that oxidized methylene blue injected into the subcutaneous

which, when complete, results in the conversion of the milk to a clear fluid, a process sometimes termed peptonization. Peptonization, however, does not follow precipitation when the microorganism does not possess casease. The bacteria producing these changes include spore-forming aerobes such as *Bacillus subtilis*, certain strains of staphylococci, *Proteus vulgaris* and others. Proteolysis may occur in heated milk in which the non-spore-forming lactic fermenters have been destroyed, leaving the more resistant spore-forming proteolytic forms.

The "Diseases" of Milk. A series of unusual or abnormal changes, sometimes called "diseases" of milk, are produced by certain bacteria which occasionally find their way into milk. "Blue milk" (*Bact. cyanogenes*), "red milk" (*Bact. prodigiosus*, *Bact. erythrogenes*, et al.) and "yellow milk" (*Bact. synxanthus*) are caused by the presence of various chromogenic bacteria. "Bitter milk," characterized by a bitterness that sometimes develops after a short interval, is likewise due to the products of certain microorganisms. Milk sometimes suffers from a ropy or slimy fermentation which, under most circumstances, is regarded as undesirable, although such a fermentation is intentionally produced in the manufacture of Edam cheese in Holland through the action of a particular species of streptococcus.

The *inert* bacteria are those which produce no visible change in milk. These include certain non-pigment-forming bacteria from water and other sources, and, in addition, most of the pathogenic bacteria that find their way into milk. Such dangerous contamination, then, is inapparent without bacteriological examination.

The Pathogenic Bacteria from External Sources. The multiplicity of sources of contamination results in the heterogeneous bacterial flora that may be found in milk. It is important from the hygienic view that, in addition to microorganisms of soil and water, the bacteria carried by man have relatively ready access to milk. The opportunity for contamination with the bacteria of human disease is, then, ever present, not only when the milk is drawn but throughout its handling until it reaches the ultimate consumer. Contamination may take place directly, as in the case of scarlet fever and other streptococci or diphtheria bacilli from the throat and typhoid bacilli from the hands of infected individuals, or it may be indirect, as when the water used to wash the milk cans is contaminated with typhoid bacilli. In any case, the microorganisms do not simply survive, as in water, but actively multiply and may be present in huge numbers in pooled milk of which but a single part was originally contaminated.

Clearly, then, the most important factors which govern the number of bacteria which may be present in milk are, first, the kind and degree of initial contamination and, second, the temperature at which the milk is kept. The production of hygienically satisfactory milk, therefore, involves cleanliness in the first instance, and immediate cooling and storage at a low temperature in the second. Practical experience has more than adequately proved the significance of these points.

The Bactericidal Property of Fresh Milk. As indicated above, the number of bacteria present in freshly drawn milk frequently shows an initial decrease. The extent of this diminution is, perhaps, exaggerated by plate

dissolved in the tissues, and only when dissolved does it exert its injurious action. Silica crystals coated with oxide of iron are not dissolved in the tissues, and they remain inert and harmless.

The pathogenesis of the lung lesions of silicosis, and the association of this condition with tuberculosis, have been studied in numerous experiments in which dusts have been brought in contact with the lung tissue of guinea-pigs or rabbits, either by repeated or prolonged inhalation, or by the direct injection of a suspension of the dust into the trachea (Kettle 1930).

An interesting experiment was recorded by Gardner (1930). Guinea-pigs were infected, by inhalation, with a strain of tubercle bacillus of unusually low virulence, which normally produced in these animals a retrogressive type of tuberculosis confined to the lungs and bronchial glands. The infection was practically never fatal. When animals so infected were exposed for 8 hours a day to quartz dust an entirely different picture was produced. After 3 to 5 months the bacilli in the lungs began to proliferate actively. The infection spread to all parts of the lungs and to the abdominal viscera, producing large and progressive lesions. The bacilli had not become more virulent, since cultures isolated from the dusted animals produced the usual type of retrogressive tuberculosis when administered to normal undusted guinea-pigs. Vorwald and Delahant (1938) recorded not only that tuberculosis in the guinea-pig and rat developed with greater vigour in animals treated with silica, but that the immunizing effect of heat-killed tubercle bacilli was inhibited by the simultaneous injection of silica.

It was generally assumed that the silica, and the lesions it produced, sensitized the tissues to tuberculous infection. The silica, that is to say, was regarded as the primary active agent. Kettle (1934) showed that this view needed considerable modification.

Different groups of guinea-pigs were injected intratracheally with a suspension of silica alone, a suspension of avirulent tubercle bacilli alone, and a mixture of silica and avirulent

bacilli alone produced relatively little tissue reaction. The mixture of silica and avirulent bacilli produced a characteristic nodular tuberculo-silicosis, closely similar to the human disease. It would seem that the typical silicotic lesions are induced not by silica acting on a normal lung but by the joint action of silica and some bacterial infective agent; and Kettle suggested that the natural human disease should be known as infective silicosis.

Histamine and Adrenaline.—Finlley (1928) tested the hypothesis that the localization of pathogenic bacteria, or of filtrable viruses, at the site of local tissue injury might be the result of the liberation of histamine, or of histamine-like substances, from the injured tissues, leading to a localized dilatation of the capillaries and an increased permeability of the capillary endothelium. Animals of a susceptible species were inoculated intravenously with an infective agent—the virus of fowl-pox, or of vaccinia, or of the Rous sarcoma, *Staph. aureus*, *Str. haemolyticus*, or the pneumococcus, and a 0.18 per cent. solution of histamine base in buffer, given intracutaneously, and control injections of buffer alone. In all cases the lesions in the histamine sites were more frequent and more severe than in the control sites. These results exemplify a general conclusion, reached by Burrows (1932) in his monograph on the localization of infection, that damage, even relatively transient, to the vascular endothelium leads to settlement of blood-borne organisms at the site. The rôle of histamine, or other substances of endogenous origin, in promoting local antibacterial defence by increasing capillary permeability, is less clear; our own observations (unpublished) indicate that it has little effect when given intracutaneously together with the bacteria under test. The importance for defence of a free exudation, of both blood fluids and leucocytes, is, however, evident in the work of Evans, Miles and Niven (1948) on intracutaneous adrenaline, doses sufficient only to constrict the skin vessels for two hours, without subsequent damage to the tissues, impeded all leucocytic emigration for that period, and were sufficient to enhance the infectivity of simultaneously injected staphylococci by 10- to 100-fold, and of *Cl. septicum* by over a million-fold.

counts; the various antibodies are present in the milk, not only the bactericidal substances but the agglutinins as well, which, by clumping the bacteria, may bring about decreased plate counts. That freshly drawn milk is both bactericidal and bacteriostatic, albeit to a mild degree, is, however, definitely established. This activity is thermolabile, being destroyed in fifteen minutes at 75° C. and in two minutes at 80° to 90° C., and disappears a few hours after the milk is drawn.

MILK-BORNE EPIDEMICS OF DISEASE*

Disease	Outbreaks		Cases	
	Number	Per Cent of Total	Number	Per Cent of Total
Typhoid and paratyphoid fever	76	45.2	1,209	12.1
Septic sore throat and scarlet fever	57	34.0	6,812	68.2
"Gastro-enteritis"	24	14.3	1,423	14.2
Bacillary dysentery	5	3.0	411	4.1
Diphtheria	5	3.0	123	1.2
Poliomyelitis	1	0.6	11	0.1
Totals	168	100.0	9,989	100.0

* In New York State exclusive of New York City as reported by Dublin, Rogers, Perkins and Graves. *Amer. Jour. Pub. Health*, 1943, 33:157.

The Determination of the Quality of Milk. By far the best index of the quality of milk is the number of bacteria it contains. The bacteria present are, in all cases, a result of contamination, and hence these micro-organisms are reliable indicators of cleanliness and care and are generally used for this purpose.

Plate Counts. The total bacterial count of a milk is, then, a reflection of its hygienic quality, and the plate count has been, and still is, widely used in the bacteriological grading of milk. Standardization of the media and of the technique is, of course, necessary for comparable results. A standard procedure has been developed under the auspices of the American Public Health Association, whose publication³ may be consulted for the details.

Standards of milk quality in terms of plate counts are in general use but vary from one locality to another. In some instances more than one grade of milk is allowable; for example, a "Grade A" milk may contain not more than

³ *Standard Methods for the Examination of Dairy Products* American Public Health Association. 9th ed., 1948. For a discussion of current practical application of these methods see Black: *Pub. Health Rep.*, 1943, 58:1605, 1641, 1681.

Mucin.—Nungester and his colleagues (1932, 1936) noted an exaltation of pathogenicity of pneumococci and streptococci when injected intraperitoneally with a suspension of hog gastric mucin. Mucin was particularly effective with bacteria which, like meningococci and gonococci, are not pathogenic to laboratory animals, and are lethal only in doses in which the bacterial toxin is active.

and Castles 1936, 1937. A similar exaltation of pathogenicity was observed in *H. influenza* (Fothergill, Dingle and Chandler 1937), *Salmonella typhi* (Rake 1935, Buttle *et al.* 1937), *H. pertussis* (Silverthorne 1938), and *Staph. aureus* (Anderson and Oag 1939), but not in *Myco. phlei*, "*B. anthracoides*," *Myco. smegmatis*, *Str. viridans* or *Bact. coli* (Anderson and Oag 1939). Human, guinea-pig and rabbit serum, and dextran are also active, as are (Miller and Castles 1936, Anderson 1935a).

The exaltation of pathogenicity produced by the mucin was not accompanied by any demonstrable change in the virulence of the bacteria, but was clearly due to some action on the defence mechanisms of the animal. The efficacy of the mucin has been variously reported as due to its viscosity and power to inhibit intraphagocytic digestion of the bacteria (Nungester *et al.* 1936, McLeod 1941); to its power to injure the defensive cells and inhibit phagocytosis (Miller and Castles 1936, McCabe and King 1951); to its interference with sensitization of the bacteria by antibody (Keefer and Spink 1938); to an anticomplementary effect (Orskov 1940); to an aggressin-like action, inhibiting the chemotactic emigration of leucocytes (Ercoli *et al.* 1945); to an increase in capillary permeability leading to fibrinous exudation that blocks further leucocytic emigration (Sandage and Stark 1949, Gale and Elberg 1952b); and to a combined anticomplementary and anticoagulant effect (Lambert and Richley 1952). In his review, which should be consulted for details of the earlier work, Olitzki (1948) concludes that the basic effect is the formation by the mucin of a protective coating on the bacteria. However, not all the effects observed can be attributed solely to such an action. The varying degrees of purity and heterogeneity of the mucin preparation used in most of these researches invalidates any firm conclusions that might be drawn. It is clear that viscosity alone is not a determining factor, though viscous undegraded Group A mucopolysaccharide (p 1240) acts like mucin, and loses its activity with its viscosity when hydrolysed (Morgan and King 1943). Landy and Batson (1949) identified the Group A mucopolysaccharide in gastric mucin as the enhancing substance which would be potentiated by adding the ash from the saline-insoluble fractions to the mucin.

The relation of the various components of mucin have been clarified by the extensive analysis made by Smith and his colleagues (H. Smith 1950, 1951, 1953, H. Smith *et al.* 1951, 1952a, b, 1953). Using *Salmonella typhi* infections in mice for assay, they identified three factors: (a) a particulate, insoluble residue (cf. Landy and Batson 1949), which could be replaced by charcoal, kaolin or talc; (b) the viscosity of the medium, which could be provided by substances like blood group mucopolysaccharide, agar, gum acacia or methyl cellulose; and (c) the essential factor, a polysaccharide containing a heparin-like substance, a blood group mucoid, a chondroitin sulphate, and another polysaccharide. Mixing the large-particulate residue, the heparin, the viscous blood mucoid and the chondroitin sulphate reconstituted an active mucin. Except for the fact that heparin is anticomplementary and anticoagulant (Lambert and Richley 1952), the mode of enhancement by these factors is still obscure.

Ionizing Radiations.—The effect on immunity of various ionizing radiations of X-rays have a three-fold bearing: on the exploration of the immune process, on the therapeutic use of irradiation in infective disease, and on the potential hazards of modern warfare. We can only briefly discuss this subject, referring the reader to the admirable review by Tahaferro and Tahaferro (1951), upon which this section is largely based.

There are many clinical reports of a beneficial action of X-irradiation in man, for example, in gas gangrene, peritonitis, and inflammatory lesions of the skin.

30,000 bacteria per milliliter as delivered, and a "Grade B" milk not more than 100,000. There is a steady movement toward more strict requirements, and the maximum number of bacteria allowed is continually reduced. More than one grade of milk is, however, undesirable in that the distribution of inferior milk is allowed, and, when possible, a single standard is preferable. In Chicago, for instance, there is but one grade of milk, and it must not contain more than 10,000 bacteria per milliliter as delivered to the consumer.

*Microscopic Counts.*⁴ Besides the ordinary macroscopic colony count obtained by plating, a microscopic method devised by Breed is of value for many purposes. In the Breed method milk is taken in a capillary pipette discharging 0.01 ml. and is dried over an area of one square centimeter on a glass slide. After washing out the fat with xylol and fixing with alcohol, the film is stained with methylene blue. The number of bacteria per square centimeter is estimated by counting a carefully measured area. The ratio used in comparing the microscopic count with the standard plate count is 4:1, although it is recognized as inaccurate and variable. This direct microscopic count does not involve incubation and has proved of special value in judging the quality of fresh milk as delivered to milk-receiving stations.

Methylene Blue Reduction. If a small amount of methylene blue is added to milk and the mixture incubated, the dye will, in time, be reduced to the colorless leuco base. Although freshly drawn milk has some small power to reduce this and other dyes, the reduction is, for all practical purposes, a consequence of the metabolic activities of contained bacteria. There is, then, a direct relation between the length of time required for reduction and the number of bacteria present, and the measurement of this reduction time provides a useful approximation of the quality of a milk. The method is best adapted to raw milk and has the great advantage that no special apparatus or skill of the operator is required. Although milk cannot be stored for more than a few hours in this way, it is generally agreed that milk which decolorizes in two hours is poor in quality, while that which does not decolorize in eight hours is excellent. Resazurin has been used to some extent in the place of methylene blue.

Bacterium Coli Count. It may be assumed that since *Bact. coli* may be used as an index of the sanitary quality of water, it could occupy a similar position with regard to milk. It will be recalled, however, that water contamination of sanitary significance is fecal, whereas this is not the case with milk; *Bact. coli*, for example, would not be associated with the presence of the diphtheria bacillus in milk. Furthermore, since the great majority of market milks contain cow feces, the estimates varying from 60 to 100 per cent, *Bact. coli* in milk is probably most often of bovine origin. Although the *coli* count has been used widely in the past, the differentiation of these microorganisms is not worth while except under special circumstances that warrant it.

The Isolation of Pathogenic Bacteria. In contrast to water, the isolation of pathogenic bacteria from milk is not only a practical but often a desirable procedure. The presence of tubercle bacilli, for example, can be conclusively shown only through the isolation of these microorganisms, in this instance usually by guinea pig inoculation. Other pathogens, such as hemolytic strepto-

⁴ For a general discussion see Brew and Breed: Amer. Jour. Pub. Health, 1945, 35-683.

milder irradiation would in some circumstances stimulate one or more of the defence mechanisms of the body; but the varied results of attempts to elicit and to analyse this beneficial response to X-rays indicate that we are still far from defining those circumstances.

The Influence of Hormones on Resistance to Infection.

There is little doubt that resistance varies with different states of hormonal balance in the animal body, but the data are for the most part too confused to yield any clear picture of the hormonal effect. The hormonal variations that have received greatest attention in connection with resistance to infection are those of the sex hormones and, within recent years, of the steroid hormones of the adrenals. It is not our purpose to review the literature of sex incidence and mortality in infective disease, or to consider the variations in resistance that accompany puberty, menstruation, pregnancy and sexual senility; or to discuss in detail the evidence for the part played by the adrenal cortex in maintaining or promoting defence reactions to infective microbes; but to give some examples from experimental work illustrating the kind of hormonal effects that have been observed.

Schütze, Gorer and Finlayson (1936) recorded a definite, but inconstantly observed superiority of female mice over male mice in their resistance to infection with *Salm. enteritidis* and *Salm. typhi-murium*; but a male superiority in resistance to infection with *Past. muriseptica*. Watson, Wilson and Topley (1938) also noted that female mice were more resistant than males to the epidemic spread of infection with *Salm. typhi-murium*. There are a few experimental studies of the effect of the female sex hormones on resistance. Rosahn, Hu and Pearce (1936) found that male rabbits were less resistant to vaccinia infection than female, and non-pregnant females less resistant than pregnant females. Jungeblut and his colleagues reported that poliomyelitis virus was inactivated *in vitro* by a preparation from the urine of pregnant mares and by the serum of the same animals. They were unable to obtain any clear evidence of protection by various hormones in experimental poliomyelitis of the monkey (Jungeblut, Meyer and Engle 1934, Jungeblut and Engle 1934). Aycock (1936, 1940, Curley and Aycock 1946), proceeding from the generally held opinion that pregnancy in women predisposes to paralytic poliomyelitis, investigated the effect of oestrogens on immunity in monkeys and mice. Oestrogens protected both normal and castrated monkeys against an otherwise fatal dose of poliomyelitis virus, an effect he attributed in part to the thickening and proliferation of the site of injection—the nasal mucosa—under the influence of the hormone. His observation (Foley and Aycock 1945) that oestrogens decreased the resistance of mice to intranasal instillation, but not to intracerebral injection of the virus, is consistent with this view. Knox (1950) studied the resistance of mice during the whole course of pregnancy and observed a gradually increasing susceptibility to the virus as gestation proceeded, the mice reverting to normal resistance within a few days of delivery. An oestrogen effect on the permeability of the mucous membranes cannot have been the sole reason for this result, because the mice became equally susceptible to oral or intravenous virus. The effect of the hormone is evidently complex depending not only on the state of the animal, but on the nature of the pathogen; thus, oestrogens are reported to decrease, not increase, the susceptibility of mice to *Str. pyogenes* (Foley and Aycock 1944).

A change in a mucous membrane may be responsible for the curative action of oestrin in gonorrhœal vaginitis in children. The cells of the immature vaginal mucosa are poor in glycogen; with the onset of puberty, or with the administration of oestrin, they become rich in glycogen, and superficial keratinization occurs. In the oestrin-treated child with vaginitis, the pH of the vaginal contents shifts from about 7.0 to 4.5-6.0, the

cocci, *Brucella*, etc., may be isolated by culture. The isolation of these bacteria is not, of course, a routine procedure but is frequently carried out when an outbreak of disease is suspected of being milk-borne. The details of the methods employed may be found elsewhere.³

Cell Count and Sediment Test. The number of leucocytes present in a sample of milk and the amount of contained dirt that can be strained out through a standard cotton disc are frequently of value in the assay of milk quality. Excessive numbers of leucocytes are present in the milk from infected udders, and when their presence is noted in Breed smears, mastitis is suggested. The amount of sediment that a sample of milk contains is, of course, an index of the extent to which it has been contaminated and is often, though not necessarily always, correlated with the bacterial count.

The Hygienic Control of Milk. The sanitary quality of milk may be controlled in one or both of two ways: in the first instance, by preventing to a considerable degree the contamination of milk and the multiplication of contained bacteria; and, in the second, by destruction of bacteria, the pathogenic forms in particular, already present in the milk.

Inspection. The periodic inspection of dairy farms is carried out by the local board of health in many places, and, although it does not insure the absence of pathogenic bacteria from milk, it is effective in increasing cleanliness and reducing the numbers of bacteria. A score card is frequently used, and a given dairy farm is rated according to a system of points.

Certified Milk. One of the earliest attempts to avoid the dangers of milk-borne infection was the elaboration of methods designed to safeguard milk at every step in its production, collection and distribution. To this end "Medical Milk Commissions" were established in a number of localities in the United States, usually under the auspices of the local medical society. Milk conforming to certain standards is certified by such a commission to be of high quality. The regulations, which are generally excellent, deal with such matters as the cleanliness of barnyard and dairy; the purity of the farm water supply; the proper sterilization of utensils; and the health of the cows and of the milkers. The bacterial content is limited to 10,000 per milliliter and the milk must be delivered within thirty-six hours.

Certified milk is undoubtedly safer to use than milk collected and transported without suitable supervision, and the work of the milk commissions has done much to improve dairy conditions in many parts of the country. At the same time, raw milk, certified or not, can never be regarded as protected against all chances of contamination; the difficulty—not to say impossibility—of making sure that no typhoid carriers and no persons suffering from a mild case of diphtheria or scarlet fever are ever employed in a dairy is, of course, self-evident. Outbreaks of diphtheria, paratyphoid fever and other diseases have, in fact, been traced to certified milk. For this reason, as well as because of the relatively high cost of production, the use of certified milk remains limited.

Pasteurization. The process of destroying pathogenic bacteria in milk is by far the most satisfactory method of controlling milk-borne infection. The use of a temperature high enough to kill most microorganisms but not so high as to produce radical alterations in the substance heated was first applied by Pasteur to preserving wines without destroying their original flavor or bouquet. Al-

the clinical observations because, as Kass and Finland (1953) point out in their concise review of the subject, the human diseases affected are mostly those without an established infective aetiology, for which no experimental laboratory analogue is known. Nor do they greatly illuminate the function of the adrenal cortex in defence, except to establish that the cells concerned in defence and antibody formation, like many other cellular and biochemical activities of the body, can be modified by excess or defect of the cortical hormones.

The adrenal cortex, unlike most other endocrine centres, is peculiarly and quickly responsive to various stresses. A single stress results in an outpouring of steroid hormones, with characteristic cellular and biochemical changes in the blood and an increase in general resistance of the animal followed by a period of "exhaustion" of the gland. Selye (1950) developed a concept, the "stress" or "adaptation" syndrome, to embrace not only acute reactions, but chronic states where there may be a long continued stage of exhaustion, accompanied by pathological changes in the tissues, which predispose the animal to many diseases, including infections. It is doubtful how far this concept is applicable to all infections, excepting in so far as any manifest infection may be regarded as a disturbance of the adaptive mechanisms of an animal—a disturbance which the adrenals may share in, but not necessarily affect in any decisive way.

There is already a vast literature, both clinical and experimental, about these hormones, and we can cite only a few examples of experimental work, referring the reader to Kass and Finland (1953) for a wider survey.

The cortisones and corticotrophin usually have similar effects, and we shall consider them together. But they differ in one important respect: the second can release only the available endogenous corticosteroids, whereas doses of cortisone can be given that are far in excess of the immediately available endogenous hormones. We shall not therefore expect their action always to be similar.

In experimental infections, the positive results are usually obtained only with pharmacological doses that induce a state of "hypercorticism," characterized among other things by depletion of lymphoid tissue, depression of the reticulo-endothelial clearing mechanisms and of the capacity to form granulation tissue. It is only in the suppression of certain hypersensitive reactions (Chapter 51) that small doses are consistently effective. The large doses, almost without exception, impair the resistance of laboratory animals. Thus in cortisone-treated mice (Hart and Rees 1950), guinea-pigs (Spain and Molonut 1950), and rabbits of a genetically resistant strain (Lurie *et al.* 1951), the lesions of tuberculosis are more extensive, more widespread and less well localized than in control animals. Friedlander (1951) recorded an increase in the incidence of arthritis and death in mice infected with *Str. viridans*, and Mogabgab and Thomas (1952) in the mortality of rabbits infected subcutaneously with *Str. pyogenes*; in the latter, death was associated with bacteraemia, and there were few clinical signs of infection. Cortisone changed mild brucella infection in mice and guinea-pigs into a fulminating and fatal infection, it had no effect on chronically infected animals, presumably because there was an associated high degree of antibody immunity (Abernethy and Spink 1952). It also lights up latent disease; LeMaistre and Tompsett (1952) observed that 82 per cent. of rats from an otherwise healthy stock developed a fulminating *C. murum* infection during cortisone treatment, and Gledhill and Rees (1952) described a spontaneous streptococcal disease in cortisone-treated laboratory mice.

In rabbit syphilis the granulomatous reactions are depressed and the multiplication of spirochaetes facilitated (Turner and Hollander 1950). A similar numerical increase in the pathogen is demonstrable in chick embryos infected with mumps virus (Kilbourne and Horsfall 1951). Among virus diseases we may cite an enhancement of vaccinia lesions in the guinea-pig (Khigman *et al.* 1951b), and in the mouse an enhancement of poliomyelitis (Findlay and Howard 1952), Japanese B encephalitis (Vollmer and Hurlblut 1951), and in-

though still widely used in connection with bottled beer and wines, the process of pasteurization is now used chiefly for the treatment of milk.

The temperature to which milk is raised and the time for which it is held there are, of course, dictated by the heat resistance of the bacterium to be killed. From the beginning attention has been directed primarily toward the tubercle bacillus, and it has been found that this bacterium is killed by exposure to a temperature of 140° F. for twenty minutes. In practice, then, a temperature of 142° to 145° F. for a period of thirty minutes provides an adequate margin of safety, and these are the requirements that are usually specified.⁵ It should be noted that the technical aspects of such treatment of milk on a large scale are of prime importance, taking the form of prevention of foaming, proper design of valves to prevent "cool pockets" and dead ends, and the like. It might be supposed that a higher temperature or longer holding time resulting in a greater margin of safety would be desirable, this is, however, not the case, for if either the time or the temperature is increased alterations take place in the physical state of the milk in which the fat is dispersed into smaller globules and will not rise to the top on standing. Such a disturbance of the "cream line" is, of course, of esthetic rather than sanitary significance.

Considerably higher temperatures and a short holding time have been and still are used to a limited extent. In the so-called "flash" process the temperature is raised to 160° F. and maintained for fifteen seconds, then the milk is immediately chilled, as it is in the "holding" process discussed above. Both time and temperature are difficult to control accurately in the flash process, and this fact, together with the "cooked" taste imparted to the milk, has severely limited the use of this method.

The plate count of milk is tremendously reduced by pasteurization, for not only are the pathogenic bacteria, such as the tubercle bacillus, *Brucella abortus*, streptococci and the like, destroyed, but the majority of other bacteria present as vegetative cells are killed also. The efficiency of the process may, then, be measured in terms of bacterial destruction and, in the last analysis, must be measured this way. Very recently, however, a test has been devised which apparently gives an accurate measure of the efficiency of pasteurization as carried out in practice. The *phosphatase test* is based upon the presence of the heat-sensitive enzyme phosphatase in milk. Since 96 per cent of the enzyme is destroyed by heating to 143° F. for thirty minutes, the amount remaining in a pasteurized milk may be used as an indicator of the pasteurization as carried out. The enzyme liberates phenol from phosphoric-phenyl esters and, as originally proposed, the test consisted of the addition of disodium phenol phosphate and Folin's reagent, incubating eighteen to twenty-four hours, and reading the blue color developed. A variety of modifications has, however, been

has become

formers, aerobic and anaerobic, certain streptococci are able to survive the pas-

⁵ Amer. Jour. Hyg., 1927, 7:147.

⁶ For discussion and references see the appendix of *Standard Methods for the Examination of Dairy Products*, loc. cit.

⁷ Burgwald. Jour. Dairy Sci., 1942, 25:295.

in the breakdown of sulphonamido-crysoidin (Prontosil) to yield active sulphanilamide. The hypothesis is generally acceptable on the grounds that (a) for a given infecting agent, *in vitro* activity broadly parallels *in vivo* activity, and (b) the activity of a given drug varies with the species and often with the strain of the infecting organism. If there was a direct stimulation of the body defences, we should expect a greater similarity of action in various infections than is actually the case. The recorded discrepancies between *in vivo* and *in vitro* activity of any one drug and a given bacterium (see, for example, Buttle *et al.* 1937, Gley and Girard 1937) do not necessarily invalidate the hypothesis, for it is seldom possible to make a precise comparison of the concentrations of drugs and drug-antagonizers, and of bacteria, in the test-tube and the tissues, or to assess the contribution made by the tissues to the antibacterial effect.

Sulphonamides.—Various *in vivo* sulphonamide effects on bacteria have been postulated. Capsular degeneration of pneumococci and streptococci was observed by Levaditi and his colleagues (1935a, 1939), by Telling and Oliver (1938) and by Whitby (1938), though Long and Bliss (1937a), Gay and Clark (1937) and Lyons and Mangiaracine (1938) did not observe it in streptococci, nor Fleming (1938b) in pneumococci. Capsular degeneration, it should be noted, may take place with the ageing of a culture of capsulated organisms, and may be distinct from the loss of a capsule that in some cases accompanies $M \rightarrow S \rightarrow R$ variation (Chapter 9). A more commonly reported change is swelling, elongation and distortion of the bacteria, seen, for example, in meningococci (Levaditi and Vaisman 1936) and streptococci (Gay and Clark 1937, Long, Bliss and Feinstone 1939). These "degenerative" changes in the streptococci were not associated with a loss of virulence, though there was a temporary variation from the mucoid to the "matt" colony form. According to Lyons and Mangiaracine (1938) and Lockwood (1938) the sulphonamides induce in streptococci changes that make them more susceptible to phagocytosis. Sulphonamides will induce a mucoid \rightarrow "smooth" transformation *in vitro*, with an associated loss of virulence, but the rate of variation is apparently too slow to be operative in the cure of acute infections (Hadley and Hadley 1941). McKinney and Mellon (1941) isolated relatively avirulent variants of pneumococci from mice treated with minimal curing doses of sulphapyridine. Hartmann (1944) was unable to detect any change in the cultural characteristics of pathogenic *Staph. aureus* during the local treatment of staphylococcal lesions in the rabbit with various sulphonamides.

Sulphonamides inhibit the action of streptococcal hæmolysins and leucocidins *in vitro* (Levaditi and Vaisman 1935a) and in bone marrow culture (Osgood and Brownlee 1938). Huntington (1938) observed a parallel inhibition of hæmolysin formation and growth in streptococci, and King, Henschel and Green (1938) an inhibition of the hæmolysin by concentrations that did not inhibit growth. The suppression, selective or not, of certain synthetic powers by an antibacterial agent is, as Garrod (1938) points out, to be expected, and is brought about by antiseptics as well as chemotherapeutic agents. There is some evidence, however, of a direct antitoxic effect of sulphonamides. The oral administration of 4-nitro-4'-aminodiphenyl sulphone and to a less extent, of sulphanilamide was found by Levaditi and Vaisman (1937, 1938) to protect mice against intraperitoneal injections of the endotoxins of *N. gonorrhææ*, *N. meningitidis*, *Past. avicida*, *Sh. flexneri*, *Sh. dysenteriae*, and *Salm. typhi-murium*. Carpenter, Hawley and Barlour (1938) demonstrated a similar *in vivo* protection against gonococcal and pneumococcal endotoxins, and against staphylococcal toxin and certain clostridial toxins. Hutner and Zahl (1942) confirmed the protective action against *Salm. typhi-murium* endotoxins (but see Buttle *et al.* 1937). The inhibition of exotoxins was not confirmed by other workers (Levaditi and Vaisman 1938, Gross *et al.* 1938, Bayliss 1940, Rigdon and Freeman 1940).

There is little unequivocal evidence for a direct action of sulphonamides on the cellular defences of the animal. Mixtures of defibrinated or heparinized blood with sulphonamides

teurizing temperature. These are the lactic acid formers rather than the pathogenic forms, and pasteurized milk sours in the ordinary way on standing, though a longer period of time is required—i.e., its keeping qualities are improved. If higher temperatures are used, 180° F., the lactic acid bacteria are killed and proteolysis occurs as the milk spoils. Thermophilic bacteria are frequently present in pasteurized milk in great numbers, for the temperature of pasteurization is an incubation temperature for these microorganisms. The slight acid metallic taste occasionally noticeable in pasteurized milk is often attributable to their biochemical activity.

It must be emphasized that the process of pasteurization, while effective in destroying pathogenic bacteria present in milk and, incidentally, increasing its keeping qualities, is not to be regarded as an excuse for the marketing of dirty and highly contaminated milk. There is some evidence, for example, that the growth of enormous numbers of bacteria, albeit nonpathogens in the usual sense, is associated with summer diarrhea of infants. In most cases, therefore, sanitary regulations specify not only the allowable number of bacteria in pasteurized milk as delivered but also an upper limit for raw milk which is to be pasteurized.

The Regulation of Milk Quality. The application of appropriate methods of rendering and keeping milk satisfactory from the hygienic point of view is, essentially, a social and legal problem rather than a scientific one. To this end appropriate ordinances are more and more generally incorporated into the legal structure of the community, and these, when adequately enforced, produce a marked reduction in milk-borne disease. A standard form of ordinance developed by the United States Public Health Service⁸ had in 1938 been adopted by more than 800 American cities. In any case, the grading and pasteurizing of milk has become general in the United States. A survey⁹ has shown that about half the cities of over 1000 population grade milk and permit the sale of one grade of raw milk and one grade of pasteurized milk; of the total volume of market milk about 74 per cent is pasteurized, 99.4 per cent is from tuberculin-tested herds, and 35 per cent from abortion-tested herds. In general, sanitary regulations are somewhat more strict and more rigorously enforced in the large cities. In Chicago, for example, all milk is pasteurized, including certified milk, and this regulation is rigidly enforced.

Such practice has resulted in marked reductions in the number of bacteria in market milk. In 1901 the bacterial content of market milk in New York City varied from 300,000 in the coldest weather to 5,000,000 during the summer months; in Chicago (1904) the counts ranged from 10,000 to 74,000,000, and in Boston (1892) averaged 4,500,000. The incidence of milk-borne disease has correspondingly decreased, for example, in 1907–1915 there were in Massachusetts 2215 cases of typhoid fever which were traced to milk, but in 1919–23 only 297.

Milk Products. Various foods made from milk, such as ice cream, butter, cheese and the like, are potential vectors of disease when made from milk contaminated with pathogenic bacteria. The bacteria tend to die out upon stor-

⁸ Pub. Health Repts., 1926, 41.1604.

⁹ Fuchs and Frank Pub. Health Bull. No. 245, 1938, also Pub. Health Repts., 1942, 57:228.

(Loewenthal 1939); in streptococcal infections of mice (Behrens 1938, Loewenthal 1939); in experimental staphylococcal infection (De and Basu 1938); in meningococcal infection of mice (Branham and Rosenthal 1937, Branham 1940); in *Salm. typhi* infections of 10-day chick embryos (Weil and Gall 1941); and to a slight extent in *H. pertussis* infection of mice (Bradford and Wold 1939). Colebrook and Moxted (1940) confirmed the findings with regard to the bactericidal power of the blood, but found only a feeble synergy in experimentally infected mice, and that only when sub-optimal doses of sulphonamide were employed.

Macleod, Rogers and Fleming (1939) found that the survival rate of mice and rabbits infected with pneumococci was increased if the animals were actively immunized at the time of the sulphonamide therapy with a single dose of pneumococcal vaccine (see also Chapter 74).

Synergy of this kind also occurs in the treatment of clostridial infections. Under careful controlled experimental conditions, Henderson and Gorer (1940) found a striking synergy of antitoxin and sulphonamide in both prophylaxis and cure of *Cl. welchii* and *Cl. septicum* infections of guinea-pigs. Singer (1940) obtained similar results; the sulphonamides used had no effect on *Cl. oedematis* infection and in this case no synergy of drug and specific antitoxin was observed. Gordon and McLeod (1941), however, employing different experimental methods, observed little curative action of sulphonamides in experimental *Cl. welchii* and *Cl. septicum* infection, and no synergy in combined drug and antitoxin treatment.

The sulphonamides are generally held to be bacteriostatic *in vivo*, restraining bacterial growth within limits that enable the defence mechanisms of the body to eliminate the invader. Some idea of the value of such restraint will be evident from the following calculation. If we assume a division of the parasite every 20 min. and a survival of all daughter cells, in 12 hours a single cell has multiplied to 2^{35} (about 10^{11}), whereas even a partial bacteriostatic effect that reduced division to once every 40 min. would limit the number at 12 hours to 2^{17} , i.e. to a number about 250,000 times 2^{-18} less than that of the uninhibited organisms.

There is thus little evidence of peculiar *in vivo* effects of the sulphonamides on the infecting organism; and little evidence of antitoxic effects, or of direct stimulation either of the non-specific or specific antibacterial mechanisms of the host, at least to a degree likely to make a significant contribution to defence.

Antibiotics.—The same conclusions apply to antibiotics, with certain differences dictated by differences in the mode of action. Some, like the sulphonamides, may act as bacteriostatics, particularly in low concentrations, and depend for their total effect on the co-operation of the tissues; others are directly bactericidal. Among these is penicillin, to which bacteria are most susceptible when they are actively dividing (Chapter 6).

The relation of bactericidal and bacteriostatic action to the host's defences is well illustrated in the papers of Eagle and his colleagues, to which reference should be made for details of earlier work on the subject. In an analysis of the optimal conditions for penicillin therapy, Eagle found that, whereas the number of streptococci or pneumococci at the site of an intramuscular injection in mice rose in untreated animals, in animals given a single dose of penicillin it declined within a few hours, at a rate proportional to the dose of penicillin, and similar to the death rate *in vitro*. Moreover, the curative single dose was proportional to the number of organisms inoculated. When the penicillin concentration in the mouse dropped below effective levels, the surviving bacteria began to multiply, but only after some hours, indicating a residual damage to the bacterial cell. This stage can be reproduced in untreated animals by injecting cocci that have been ex-

"in vitro" the cocci do not begin to multiply freely in the muscle until a bacteri-

age, of course, although it has been found that the typhoid bacillus will survive for three months or more in butter, and tubercle bacilli have been found in butter and certain quick-ripening varieties of cheese. Ice cream may serve as a vector for typhoid fever, scarlet fever and the like. Pasteurization of the mix is customary and generally at higher temperatures than those used for milk. Human infections with foot-and-mouth disease have been traced to contaminated butter and cheese, but the public health significance of these findings is problematical.

FOOD POISONING AND FOOD-BORNE INFECTION¹⁰

The diseases transmitted by milk and milk products may be disseminated by a variety of other foods, in addition to these, however, other types of illness may result from the ingestion of contaminated foods which make up that group of affections designated as food poisoning. The kinds of illness that may result from the ingestion of food may be summarized briefly.

- (1) individual idiosyncrasies;
- (2) toxemia from foods, such as
 - (a) foods naturally poisonous,
 - (b) foods into which poisons have been accidentally introduced and
 - (c) foods containing poisons of bacterial origin formed by
 - 1 *Clostridium botulinum* and
 - 2 staphylococci,
- (3) food borne infection, including both
 - (a) bacterial infections, such as
 - 1 typhoid fever, dysentery, cholera *et al.* and
 - 2 *Salmonella* infection, and
 - (b) parasitic infections.

It will be clear that in some instances food serves simply as a vector in the transmission of diseases such as the parasitic infections and the enteric diseases. In the remainder, however, the clinical manifestations are those associated with food poisoning proper—vomiting, diarrhea, enteritis and a greater or lesser degree of prostration. Although a number of types of food poisoning given above are not bacterial in origin, their clinical symptoms frequently are those of bacterial food poisoning, and these must be considered in any attempt to ascertain the etiology of a given outbreak, even though food poisoning is, in a majority of instances, a consequence of bacterial activity. Hypersensitivity (p 334) to a given food substance, for example, is frequently manifested as vomiting, and an outbreak confined to a family may be the result of familial tendency, similarly, the gastro-intestinal disturbances following the ingestion of naturally poisonous foods such as toadstools, or foods contaminated with poisons such as arsenic or cyanide, are often indistinguishable from those induced by some poisons of bacterial origin.

Food Poisons of Bacterial Origin. Often popularly termed "ptomaine poisoning," poisoning with food containing toxic substances of bacterial origin is very common, probably more so than is generally recognized. The term "ptomaine poisoning" is a misnomer that is both misleading and inaccurate.

¹⁰ Jordan *Food Poisoning and Food Borne Infection* 2nd ed. University of Chicago Press, Chicago, 1931. Dack *Food Poisoning*. University of Chicago Press, Chicago, 1943

the occurrence of antagonism between various pairs of antibiotics. There are reasons (p. 207) for thinking that it is not as important in clinical practice as it appears to be from laboratory investigations. For the therapeutic implication of these phenomena we refer the reader to the papers of Jawetz and Gunnison (1952) and Garrod (1953).

The relation of antibiotics to specific immunity is like that of the sulphonamides, with differences imposed by the more effective direct bactericidal action of certain antibiotics. Harrison (1946), for example, compared sulphonamides and penicillin in rabbits receiving intradermal injections of lethal doses of pneumococci. Penicillin abated the infection when given 4 hours after the cocci; there was no local lesion, and neither agglutinins nor immunity to later re-infection developed. With curative doses of penicillin given 24 hours after the cocci, the lesions resolved, and a high degree of specific immunity developed. Sulphapyridine, on the other hand, given at 4 or 24 hours, was slower in controlling the infection, but a certain immunity developed afterwards. The immunity, however, was less in the "24-hour" group than in the "4-hour" group. It appears that the early penicillin destroyed the cocci before they grew sufficiently to provide an adequate antigenic stimulus, and the late penicillin quickly killed the more numerous cocci, thereby providing a good antigenic stimulus. The early sulphonamide, on the other hand, allowed growth enough to provide a sufficient stimulus, but after later sulphonamide the antibody response was to some extent neutralized by large amounts of capsular substance elaborated as a result of the more extensive and continuing infection. Harrison concluded that penicillin was less dependent on the immune response than the sulphonamide.

According to Miller and Foster (1944) and Kelly and Schnitzer (1945), the combined effect of penicillin and specific antibody is synergic. However, Browning and Calver (1947), working with *Staph. aureus* infection in sulphathiazole- and penicillin-treated mice, concluded that the beneficial effect they observed with α -antitoxin was additive; and Treffers and Muschel (1954) clearly demonstrated that the combined bactericidal action of antibody and complement and chloramphenicol on *Salm. typhi* *in vitro* was not synergic, but purely additive. As regards the impairment of antibody response during antibiotic treatment, several observers have recorded that the rapid elimination of streptococci from the lungs in infection of man reduces the subsequent antibody response (e.g. Rantz *et al.* 1946, Kilbourne and Loge 1948). In the experimental animal, in addition to the work of Harrison discussed above, we may note examples of a similar reduction of the immunizing stimulus of infection in the early penicillin treatment in rats and guinea-pigs infected with pneumococci (Skinses 1948); and in mice infected with *Br. tularensis* and given streptomycin (Chapman *et al.* 1949). Speck and Jawetz (1952) treated mice with penicillin and aureomycin 24 hours after infection with *Str. pyogenes*, and found that the immunity of survivors to reinfection 16 days later was greater in the animals receiving the less effective antibiotic, which in this case was penicillin.

The alternative explanation of a direct suppression of antibody synthesis by the antibiotic has been considered. Green, Wohl and Waife (1951) could demonstrate no suppression by penicillin of the *H*-agglutinin response in man to typhoid vaccine. However, using an indirect indicator of antibody formation, Stevens (1953) reported that the response of rabbits to a protein antigen was depressed by the continued administration of dihydrostreptomycin, terramycin, penicillin and aureomycin. Some rabbits in each group were resistant to this action, and particularly to the last two antibiotics.

Two of the possible effects of chemotherapy, the emergence in the infected animal of drug-resistant strains, and the suppression of the infecting agent before it has grown to the point where it provides an effective antigenic stimulus, have implications not only for the treated individual, but for the herd. The widespread use of an antibiotic in a community might lead in the first case to the establishment of endemic infection with drug-resistant bacteria, and in the second, to the perpetuation of a disease prevalence that in the normal course of events would decline. recovered individuals in the community. emergence of penicillin-resistant staphylococci.

The organic bases such as putrescine, cadaverine, methylamine and the like, which have been called ptomaines and which result from the bacterial decomposition of protein, are not toxic when given by mouth. Neither are other decomposition products toxic *per os*. While a partially decomposed food may be esthetically unattractive, the innocuous nature of the products of decomposition is obvious when one considers the advanced state of decomposition reached by some cheeses. Toxicity is, on the contrary, attributable to the presence of substances synthesized by the bacteria whose presence may or may not be associated with obvious evidence of decomposition of the food substance.

Botulism. Of the toxic substances formed by bacteria in food the most powerful is the toxin of *Clostridium botulinum* (p. 593). In the United States canned foods provide the anaerobic conditions necessary for the germination of botulinus spores which have survived processing, while in Europe meat products, particularly the larger sausages, have been more frequently involved. The microorganism never infects man under natural conditions, and the disease is an intoxication resulting from the ingestion of preformed toxin present in the food substance. The disease is not of great public health importance—about 2000 cases have been reported in all—and the outbreaks are generally limited in scope, often being confined to a family circle of a few individuals. The case fatality is high, however, probably 60 to 80 per cent, and when outbreaks do occur they attract considerable attention. This type of food poisoning differs from the others in that the symptoms are not necessarily gastro-intestinal, the toxin acts upon the peripheral nerves and the symptoms are those resulting from such damage. The use of botulinum antitoxin is discussed elsewhere.

Since both anaerobic conditions and a period of incubation are required for the growth of *Cl. botulinum* and the formation of toxin, fresh foods are not involved in this type of food poisoning. The toxin is destroyed by heat, hence freshly cooked foods do not contain it. Canned foods and certain meat products which are consumed either cold or after simply warming may contain botulinum toxin if the spores of this bacterium were originally present and not destroyed. Commercial processing as now employed in this country is adequate to destroy botulinum spores, and at the present time outbreaks of botulism are frequently attributable to the consumption of home canned, insufficiently heated foods. The presence of toxin is not necessarily associated with obvious signs of spoilage, though it is probable that toxin-containing foods are never entirely normal in appearance, odor and the like.

Staphylococcus Food Poisoning. The marked gastro-intestinal disturbances characteristic of food poisoning are not infrequently associated with the consumption of foods containing starch thickening, such as eclairs, cream puffs, certain types of cake fillings and salad dressings, and the like. Upon bacteriological examination, the incriminated food generally is found to contain enormous numbers of bacteria, sometimes staphylococci, other times bacteria of the colon-aerogenes group, streptococci, *Proteus* and similar microorganisms.

The causal relation of staphylococci, generally, though not necessarily, hemolytic strains of the *Staphylococcus aureus* group, to food poisoning was

by the virus. The insusceptibility, however, appears to be realized at the expense of considerable damage to the nasal mucosa (Schultz and Gebhardt 1942, see also Chapter 87).

The Rôle of the Bacteriophage in Infection and Resistance

This is, perhaps, as convenient a place as any to summarize the available evidence with regard to the rôle played by the bacteriophages in infection and resistance. The relationship between the various types of phage and the bacteria that are sensitive to their action is highly specific, and is usually determined by the nature of the antigenic components situated at the bacterial surface.

The existence of living viruses, specifically attacking various pathogenic bacteria, offered an obvious possibility that they might be employed in the prevention or cure of infective disease. That they can be so employed, and are highly effective, was claimed by d'Herelle (1926); and several observers have reported favourable results in field trials of cholera phage as a prophylactic in India (see Morrison 1932). There are also various observations in the literature with regard to the use of phages as therapeutic reagents in human infections (see Krueger and Scribner 1941); but these are discrepant, and not of the kind that provide us with the evidence we need, though on the occasions when large-scale observations are made with as great a care as the circumstances permit, the results are not encouraging (see *e.g.*, Boyd and Portnoy 1944). Morton and Engley (1945a) consider that, although dysentery phage is prophylactically useful in man, the evidence for a therapeutic effect is inconclusive, and they set out criteria for more stringent tests. These include bacteriological identification of the infecting organism and proof of its susceptibility to the phage used, bacteriological proof of cure, freedom of the control cases from a naturally acquired dysentery phage; exclusion of effects due to changes in other intestinal organisms, and proof that the administered phage reaches the infecting organisms in active form. Aside from a theoretical lethal action of the phage on the infecting bacteria, it is possible that the phage filtrates are beneficial because they act as specific vaccines, since they contain the antigens of lysed bacteria; as stimulants of a non-specific immunity; or as opsonins (see Krueger and Scribner 1941). In this last connexion we may note that MacNeal, McRae and Colmers (1938) demonstrated a substantial opsonic effect when optimal quantities of specific phage were mixed with *Staph aureus* and added to human blood. Until some years ago there was on the experimental side little evidence that phage had an *in vivo* antibacterial action, except for a few observations suggesting that phages injected into an animal simultaneously with such organisms as *Salm. typhi* or *Bact. coli* had a slight protective action (Wollman 1925, Arnold and Weiss 1926, Walker 1929). For reports of successful use of phage in the treatment of typhoid fever, see Desranleau (1948) and Dhayagude and Banker (1949).

Thus there was a general failure to demonstrate any protective or curative action of phage filtrates against *Salm. typhi-murium* or *Salm. enteritidis* when tested in mice (Topley and Wilson 1925, Topley *et al.* 1925, Levy 1925, Wollman 1925, Richet and Hauduroy 1925, Bronfenbrenner and Korb 1925-26, Ebert and Peretz 1929, Greenwood *et al.* 1936). Similarly, phage filtrates had little or no protective or curative action in salmonella infections in fowls (Pyle 1926), in experimental plague in rats (Compton 1928, Hale 1931). Nor was there any experimental evidence that the presence of phages in the intestines of mice of a phage acting on *Salm. typhi-murium* prevents the spread of natural contact

suggested in 1914¹¹ and in recent years this relationship has been definitely established.¹² These bacteria produce a soluble toxic substance, or *enterotoxin*, which gives rise to typical food poisoning symptoms in man and rhesus monkeys upon feeding and in kittens upon injection. There is considerable doubt as to the specificity of the kitten test, however, for it has been shown that the staphylolysins will cause vomiting on injection into these animals.¹³ The incubation period in man is short—two to six hours—and the case fatality nil, with complete recovery in twenty-four to forty-eight hours. The nature of the enterotoxic substance is unknown, although its formation by staphylococci appears to be favored by the presence of starch. A period of incubation is necessary for its elaboration by the bacteria, and in outbreaks of this type of food poisoning it is always found that a period of time, generally not less than eight hours, has elapsed between the preparation and the consumption of the food. This type of food poisoning is quite common and a number of well authenticated outbreaks have been reported.¹⁴

Whether or not bacteria other than the staphylococci form similar enterotoxic substances is open to question. The fact that the same incubation period and clinical symptoms are observed when epidemiological evidence incriminates the other bacteria noted above suggests that enterotoxin may be formed by them. Streptococci are occasionally incriminated in food poisoning outbreaks with evidence of enterotoxin formation,¹⁵ and *Bacterium aerogenes*, *Proteus* and similar organisms have been found under much the same circumstances. There is some experimental evidence¹⁶ indicating that the ability to form substances irritating to the human alimentary tract may be one that is possessed by a variety of bacteria, but as yet this is not definitely established.

Food-Borne Bacterial Infections. The food-borne bacteria infections are of two general types. The one consists of those diseases which are transmitted by a variety of vectors of which food is but one and whose clinical symptoms are not those usually associated with food poisoning. Such, for example, are typhoid and paratyphoid fevers, dysentery, cholera and other enteric infections. The second type of infection is that with bacteria of the *Salmonella* group, in which the incubation period is short, the gastro-intestinal disturbance is of short duration, a day or two, and the symptoms are typical of food poisoning. There is some crossing between the two types, for paratyphoid B bacilli (*Salmonella paratyphi* B) may produce either the typical food poisoning response or paratyphoid fever.

The first group ordinarily comprises the so-called "residual typhoid," for example, which remains in spite of hygienic measures. The second group, on the other hand, is the first group ordinarily food-borne and, to a lesser extent, water-borne. The second group, on the other hand, plays an important part in maintaining some of these diseases in endemic form.

¹¹ Barber. *Philippine Jour. Sci.*, 1914, 9:515.

¹² Dack, Cary, Woolpert and Wiggers. *Jour. Prev. Med.*, 1930, 4:167; Jordan: *Jour. Amer. Med. Assn.*, 1930, 94:1648.

¹³ Fulton: *Brit. Jour. Exp. Path.*, 1943, 24:65.

¹⁴ Cf. Jordan and Burrows. *Amer. Jour. Hyg.*, 1934, 20:604.

¹⁵ Foley, Wheeler and Gettings. *Amer. Jour. Hyg.*, 1943, 38:250.

¹⁶ Jordan and Burrows. *Jour. Inf. Dis.*, 1935, 57:121.

induced by the action of phage, but they appeared to have little virulence. On the other hand, strains of *Salm. typhi-murium*, isolated from mice that had died of infection in spite of the presence of phage in the tissues, proved just as sensitive to that phage as the bacteria originally inoculated (Topley and Wilson 1925, Topley *et al.* 1925, Greenwood *et al.* 1936).

Blood, serum, bile, white cells, tissue debris can all inhibit lytic action of phage

may be (see Zdansky 1921) that the cells or colloids of the body fluid exert *in vivo* an inhibiting effect on phage lysis similar to the inhibition exerted *in vitro* by high concentrations of gelatin or agar (Bail 1922, Doerr and Berger 1922, Otto and Munter 1923, Bronfenbrenner and Korb 1925). This suggestion is supported by the observation that some of the most hopeful results of bacteriophage therapy have been obtained in cholera—a disease in which the infecting vibrios are multiplying freely in the watery contents of the intestine.

In any case, until we know more than we do of the factors that prevent any but high concentrations of phage from exerting any protective effect in the tissues, we cannot justifiably conclude that it is impossible so to adjust the conditions that a large part of the *in vitro* activity might be made available for therapy in the infected animal.

SUMMARY

(1) There is no doubt that variations in diet are associated with variations in resistance to infective diseases. Starvation to the point of inanition, resulting either from lack of protein or calorie in the diet, or from a low food intake following loss of appetite due to depletion of certain vitamins, decreases both non-specific resistance and the capacity to synthesize antibodies; and lesser degrees of malnutrition probably have a similar though lesser effect.

(2) Depletion of protein is clearly a cause of lowered resistance. Depletion of Vitamin A, especially in the period of rapid bodily growth, is associated with a lowered resistance, but there is no good evidence that depletion of Vitamin D has any significant effect on resistance. Quite apart from any accompanying general malnutrition, depletion of several of the water-soluble vitamins—those of the B-complex, and Vitamin C—decreases resistance and antibody formation.

There is no reason to suppose that the dietary factors which affect resistance are confined to the known vitamins.

Whether a given depletion is effective in this respect depends on the species of host and of parasite, and the tissue responses characterizing the particular infection. This dependence is strikingly illustrated in certain virus infections, in which a depletion apparently increases the host's resistance; presumably because virus multiplication depends on active tissue metabolism.

(3) There is no consistently good evidence that any food factor in excess of normal requirements enhances resistance to infections.

(4) The experimental evidence with regard to the influence of fatigue on resistance suggests that it is more important as a factor leading to the activation of a latent infection than as predisposing to infection *ab initio*.

(5) The data with regard to the effect of variations in the physical environment are scanty. It is certain that fluctuations in temperature and humidity

control of water and milk supplies is, in large part, food-borne. The employment of cooks and other food handlers who are typhoid carriers provides the opportunity for food infection and consequent transmission of the disease. Mary Mallon—"Typhoid Mary"—was one of the most notorious examples of a cook who was a typhoid carrier.

Of the *Salmonella* infections, one of three species is generally involved, *Salmonella typhi-murium* (*S. aertrycke*) and its varieties (*newport*, *stanley*, etc.), *Salmonella enteritidis* and *Salmonella cholerae-suis* (including *Voldagsen* and *paratyphi C*). *S. typhi-murium* is the most frequently observed, while *S. cholerae-suis* is only rarely present. The ingestion of food containing large numbers of these organisms frequently results in the typical symptoms of food poisoning. No enterotoxin substance has been shown to be formed by these bacteria, and it is probable that actual infection takes place as indicated by the somewhat longer incubation period and the finding of the bacteria in the feces. The case fatality is variable, ranging from zero to 10 per cent. Almost any kind of food may serve to carry these microorganisms, although meats and other protein foods predominate. This type of food poisoning is not common.¹⁷

Both *S. typhi-murium* and *S. enteritidis* are commonly carried by rats and mice, and it is probable that in many cases these rodents are responsible for the infection of food. In others the source of infection may be a human carrier, and in many cases of meat poisoning it has been found that the meat was from a diseased animal.

Shellfish and Disease. Shellfish, including oysters, clams and mussels, have been responsible for the transmission of enteric disease, especially typhoid fever, through pollution of areas in which they are grown or stored. They are commonly eaten in an uncooked or partially cooked condition which facilitates transmission of disease; it has been found that, while scalloped oysters, fried clams and clams in chowder are practically sterilized, steamed clams, fried oysters, oysters in stew and mussels cooked in the usual ways are not freed from coliform bacteria. The shellfish, in the course of breathing and feeding, filter large quantities of water and readily take up enteric pathogens from polluted beds or when placed in brackish waters near sewage outfalls to "fatten." Imbibed bacteria, if not ejected very soon, are passed through the gastro-intestinal tract and discharged in about five hours. In the warm season the pollution of the water of a bed or storage area is highly correlated with infection of

rapidity with which bacteria are passed through shellfish suggests the possibility that they may be cleansed of infection by storage in clean or even chlorinated water. The possibility is not only feasible but practiced, four days sufficing to practically eliminate all coliform bacteria. Essentially the same methods that are used for the bacteriological examination of water (p. 253) are applied to shellfish. The United States Public Health Service has suggested that not more than 50 per cent of the 1 ml. samples of pooled shell liquor and finely chopped tissue of 10 or more oysters, clams or mussels should show

¹⁷ Savage and White: *Food Poisoning: a Study of 100 Recent Outbreaks*. Medical Research Council Special Report Series No. 92, 1925.

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coliform bacteria in the presumptive test but the figure is a guide rather than a standard and is not inflexible.

The food-borne parasitic infections include the various flukes, tapeworms, echinococcus and round worms that infect man. In these diseases the infective stage of the parasite is often present in a food substance as a consequence of its life cycle and the mechanisms involved may be found elsewhere (See Chapter 34.)

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IMMUNITY—ANTIGENS, ANTIBODIES AND THE ANTIGEN-ANTIBODY REACTION¹

It has been common knowledge for many years that recovery from certain of the infectious diseases is accompanied by the development of an enhanced resistance, and second attacks of a disease once overcome are not common. This enhanced resistance, or immunity, is specific, *i.e.*, an individual immune to one disease may be no more than ordinarily resistant to others, and is *variable from one disease to another, some giving rise to a "solid" immunity of long duration while in others the immunity is imperfect or partial and transient.* The specific immune state supplements the complex of factors which make up non-specific resistance to infection, and in some instances even a solid immunity may be broken down by fatigue, malnutrition and similar factors which are not consistent with a state of physiological well-being. Immunity arises as a consequence of the reaction of the host to intimate contact with the parasite, or its products, within the tissues, and constitutes a last line of defense whose presence frequently prevents infection and whose development during an attack of disease determines the outcome. The reaction of the host to the invading microorganism has been studied intensively since the early days of bacteriology, and the body of knowledge so accumulated makes up the extraordinarily complex science of immunology. These studies have not only led to some degree of understanding of the phenomenon of specific resistance to infection but in addition have provided biology with a new method whose general application has been, thus far, limited.

ANTIGENS

An antigen is ordinarily but unsatisfactorily defined as any substance whose introduction into the tissues of an animal results in the appearance, after a suitable length of time, of antibodies in the blood serum and other body

¹ Much of the earlier work on immunity and related problems will be found in the following volumes: Ehrlich, *Gesammelte Arbeiten zur Immunitätsforschung*, Berlin 1904, 1908, 1910, 1912, 1914, 1916, 1918, 1920, 1922, 1924, 1926, 1928, 1930, 1932, 1934, 1936, 1938, 1940, 1942, 1944, 1946, 1948, 1950, 1952, 1954, 1956, 1958, 1960, 1962, 1964, 1966, 1968, 1970, 1972, 1974, 1976, 1978, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016, 2018, 2020, 2022, 2024, 2026, 2028, 2030, 2032, 2034, 2036, 2038, 2040, 2042, 2044, 2046, 2048, 2050, 2052, 2054, 2056, 2058, 2060, 2062, 2064, 2066, 2068, 2070, 2072, 2074, 2076, 2078, 2080, 2082, 2084, 2086, 2088, 2090, 2092, 2094, 2096, 2098, 2100, 2102, 2104, 2106, 2108, 2110, 2112, 2114, 2116, 2118, 2120, 2122, 2124, 2126, 2128, 2130, 2132, 2134, 2136, 2138, 2140, 2142, 2144, 2146, 2148, 2150, 2152, 2154, 2156, 2158, 2160, 2162, 2164, 2166, 2168, 2170, 2172, 2174, 2176, 2178, 2180, 2182, 2184, 2186, 2188, 2190, 2192, 2194, 2196, 2198, 2200, 2202, 2204, 2206, 2208, 2210, 2212, 2214, 2216, 2218, 2220, 2222, 2224, 2226, 2228, 2230, 2232, 2234, 2236, 2238, 2240, 2242, 2244, 2246, 2248, 2250, 2252, 2254, 2256, 2258, 2260, 2262, 2264, 2266, 2268, 2270, 2272, 2274, 2276, 2278, 2280, 2282, 2284, 2286, 2288, 2290, 2292, 2294, 2296, 2298, 2300, 2302, 2304, 2306, 2308, 2310, 2312, 2314, 2316, 2318, 2320, 2322, 2324, 2326, 2328, 2330, 2332, 2334, 2336, 2338, 2340, 2342, 2344, 2346, 2348, 2350, 2352, 2354, 2356, 2358, 2360, 2362, 2364, 2366, 2368, 2370, 2372, 2374, 2376, 2378, 2380, 2382, 2384, 2386, 2388, 2390, 2392, 2394, 2396, 2398, 2400, 2402, 2404, 2406, 2408, 2410, 2412, 2414, 2416, 2418, 2420, 2422, 2424, 2426, 2428, 2430, 2432, 2434, 2436, 2438, 2440, 2442, 2444, 2446, 2448, 2450, 2452, 2454, 2456, 2458, 2460, 2462, 2464, 2466, 2468, 2470, 2472, 2474, 2476, 2478, 2480, 2482, 2484, 2486, 2488, 2490, 2492, 2494, 2496, 2498, 2500, 2502, 2504, 2506, 2508, 2510, 2512, 2514, 2516, 2518, 2520, 2522, 2524, 2526, 2528, 2530, 2532, 2534, 2536, 2538, 2540, 2542, 2544, 2546, 2548, 2550, 2552, 2554, 2556, 2558, 2560, 2562, 2564, 2566, 2568, 2570, 2572, 2574, 2576, 2578, 2580, 2582, 2584, 2586, 2588, 2590, 2592, 2594, 2596, 2598, 2600, 2602, 2604, 2606, 2608, 2610, 2612, 2614, 2616, 2618, 2620, 2622, 2624, 2626, 2628, 2630, 2632, 2634, 2636, 2638, 2640, 2642, 2644, 2646, 2648, 2650, 2652, 2654, 2656, 2658, 2660, 2662, 2664, 2666, 2668, 2670, 2672, 2674, 2676, 2678, 2680, 2682, 2684, 2686, 2688, 2690, 2692, 2694, 2696, 2698, 2700, 2702, 2704, 2706, 2708, 2710, 2712, 2714, 2716, 2718, 2720, 2722, 2724, 2726, 2728, 2730, 2732, 2734, 2736, 2738, 2740, 2742, 2744, 2746, 2748, 2750, 2752, 2754, 2756, 2758, 2760, 2762, 2764, 2766, 2768, 2770, 2772, 2774, 2776, 2778, 2780, 2782, 2784, 2786, 2788, 2790, 2792, 2794, 2796, 2798, 2800, 2802, 2804, 2806, 2808, 2810, 2812, 2814, 2816, 2818, 2820, 2822, 2824, 2826, 2828, 2830, 2832, 2834, 2836, 2838, 2840, 2842, 2844, 2846, 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3844, 3846, 3848, 3850, 3852, 3854, 3856, 3858, 3860, 3862, 3864, 3866, 3868, 3870, 3872, 3874, 3876, 3878, 3880, 3882, 3884, 3886, 3888, 3890, 3892, 3894, 3896, 3898, 3900, 3902, 3904, 3906, 3908, 3910, 3912, 3914, 3916, 3918, 3920, 3922, 3924, 3926, 3928, 3930, 3932, 3934, 3936, 3938, 3940, 3942, 3944, 3946, 3948, 3950, 3952, 3954, 3956, 3958, 3960, 3962, 3964, 3966, 3968, 3970, 3972, 3974, 3976, 3978, 3980, 3982, 3984, 3986, 3988, 3990, 3992, 3994, 3996, 3998, 4000, 4002, 4004, 4006, 4008, 4010, 4012, 4014, 4016, 4018, 4020, 4022, 4024, 4026, 4028, 4030, 4032, 4034, 4036, 4038, 4040, 4042, 4044, 4046, 4048, 4050, 4052, 4054, 4056, 4058, 4060, 4062, 4064, 4066, 4068, 4070, 4072, 4074, 4076, 4078, 4080, 4082, 4084, 4086, 4088, 4090, 4092, 4094, 4096, 4098, 4100, 4102, 4104, 4106, 4108, 4110, 4112, 4114, 4116, 4118, 4120, 4122, 4124, 4126, 4128, 4130, 4132, 4134, 4136, 4138, 4140, 4142, 4144, 4146, 4148, 4150, 4152, 4154, 4156, 4158, 4160, 4162, 4164, 4166, 4168, 4170, 4172, 4174, 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fluids. With certain exceptions, the reaction is *specific* in that each antigen stimulates the formation of antibody for itself and no other antigen, and it takes place only when the antigen is a *foreign substance* to the animal into which it is injected. This characterization is of course, one that is based on what antigens do rather than what they are, for knowledge of the nature of these substances is as yet too fragmentary to permit a general definition in terms of composition and configuration of the chemical compounds which exhibit the property of antigenicity.

In general, antigenic substances are proteins, and probably all naturally occurring proteins soluble in the body fluids and containing a full complement of amino acids (the so-called complete proteins) may function as antigens. According to Wells² the presence of aromatic radicals is associated with antigenicity, proteins deficient in aliphatic amino acids but containing aromatic amino acids, such as zein, gliadin, egg albumin and casein, are antigenic, while gelatin and protamines which are deficient in aromatic radicals are not antigenic. The property of antigenicity is not destroyed by heating except as the protein is rendered insoluble by coagulation (specificity is somewhat altered), but it is lost upon hydrolysis, probably at a very early stage, although the precise point at which antigenicity disappears during hydrolytic cleavage of the molecule is not known. Antigenicity, then, appears to be a property of the intact, or nearly intact, protein molecule, and there is reason to believe that one prerequisite of antigenicity is a large molecule which may exist in colloidal solution.

Iso-antigens. It is not strictly true that antibody response is induced only by antigens foreign to the inoculated animal unless the term foreign is taken to mean not present in the circulation of that animal. It was early observed by Hektoen³ that thyroglobulin would act as an antigen in the same species of animal from which the tissue was taken, and since then it has been found that lactating goats will produce antibody to their own casein and that lens protein is antigenic even in the same individual animal. It has long been known, too, that the serum of the viper will protect against the venom of that snake as efficiently as the best hyperimmune horse serum. It is definitely established, therefore, that an animal will produce antibody against certain antigens occurring and their corresponding certain types of an general, iso-antibody titers are low.

The Human Blood Groups.⁴ It was discovered by Landsteiner⁵ that human blood could be divided into four immunological groups which represent the four possible combinations of two antigens present in the erythrocyte. The serum contains antibody (agglutinin) for the absent antigens, antigen and corresponding antibody do not coexist in the same blood. There are three systems of nomenclature for these groups (see table) of which the International

² Wells: *The Chemical Aspects of Immunity*, 2nd ed. Chemical Catalog Co., New York 1929.

³ Cf. Lewis: *Jour. Inf. Dis.*, 1934, 55:168.

⁴ See the review by Loutit: *Nature*, 1944, 153:97, and the discussion by Wiener and Kausch: *Jour. Immunol.*, 1944, 49:51.

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is by far the most widely used. Additional immunological factors are also known to be present and are responsible for a part of the observed transfusion incompatibilities; these can be avoided if donor and recipient blood are tested against one another as well as typed. These blood groups are inherited by Mendelian law and for this reason are of considerable interest in connection with the question of normal antibodies (p. 329) as well as having some forensic utility in cases of disputed paternity, etc. Blood groups occur in lower animals as well as in man, as in the rat⁶ and in cattle.⁷

The Specificity of Antigens. Closely associated with the nature of antigens is the specificity of the immunological reactions. Not only does a given antigen stimulate the formation of a specific antibody but it will react, either *in vivo* or *in vitro*, only with its own antibody or antibodies to closely related antigens. For example, the serum proteins of the higher animals, while highly specific for species, show cross reactions with the antibodies to the serum proteins of closely related species; human blood serum shows no immunological relationship to horse or rabbit serum but reacts to some degree with antisera prepared against the serum proteins of anthropoid apes and certain monkeys.⁸ Immunological differences between individuals of a single species are, of course, found in the human blood groups. The inheritance of blood groups and the correspondence of immunological relationships of species with generally

HUMAN BLOOD GROUPS

Groups			Erythrocyte antigens	Serum antibodies
International	Moss	Jansky		
O	IV	I		a, b
A	II	II	A	b
B	III	III	B	a
AB	I	IV	A, B	

accepted zoological classifications are indicative of the fundamental significance of antigenic specificity.⁹

It should be noted, however, that, although the great majority of antigenic substances are species-specific, certain antigens are found to occur in distantly related organisms. Of these the more important are lens protein (actually two proteins, α and β crystallin), which is common to a wide variety of animals, and an antigen known as Forssman antigen, heterophile antigen or, less frequently, heterogenetic antigen. Heterophile antigen has been found in the organs of the guinea pig, horse, cat, dog, mouse, chicken, turtle and several

⁶ Burhoe. Proc. Nat. Acad. Sci., 1947, 33:102.

⁷ Singh: Indian Jour. Vet. Sci. Animal Husb., 1942, 12:12.

⁸ Such immunological relationships are discussed at length by Nuttall: *Blood Immunity and Blood Relationship*. Cambridge. 1904.

⁹ See the review by Boyden: *Physiol. Zool.*, 1942, 15:109.

virus invariably resulted in a frank case of the disease, diagnosable by ordinary clinical methods, it has become increasingly clear of late years that atypical, abortive, and latent infections are very common and play an important part in the genesis of naturally acquired immunity. Even in so highly contagious a disease as measles, there is evidence to show that *not every susceptible child who is exposed to infection contracts the disease.*

Stocks (1928, 1930*a, b*), in a series of careful analyses of the relevant data, showed that the morbidity figures for measles, for chicken-pox and for German measles are inexplicable except on the assumption of immunization in the absence of diagnosed disease, or of an effective inherited immunity. That the former factor is operative can be demonstrated by comparing, during any epidemic prevalence, the attack rate among children who have previously been intimately exposed to risk without contracting the disease with that among children who have previously been less intimately exposed. The natural history of these widely prevalent virus diseases, as developed by Stocks, presents striking analogies to the natural history of diphtheria or of scarlet fever.

In other virus diseases the occurrence of atypical cases and of healthy carriers has obtained general recognition. Wickman (1907), for example, pointed out that it was impossible to account for the epidemiological behaviour of poliomyelitis without assuming the existence of latent and atypical infections; and Stocks (1932), examining the figures of a series of outbreaks, calculated that the ratio of those developing latent immunizing infections to those developing clinical attacks of the disease was probably of the order of 100 to 1 or more. The same ratio was reached by Howe (1949) in the United States of America.

These estimates, based on epidemiological data, have been largely corroborated by the finding of neutralizing antibodies in the general population

Aycock and Kramer (1930*a, b, c*), using the highly susceptible monkey as a test animal, determined the protective power of samples of serum obtained from convalescent cases and from normal persons. Some 90 per cent. of the former and just over 50 per cent. of the latter showed protective antibodies in the serum. Similar tests were later carried out on normal persons, giving no history of poliomyelitis, living in another area of the United States. Of 21 adults, 18 showed neutralizing antibodies in their blood. Brodie (1932) recorded analogous findings in Montreal. In endemic areas infection occurs at a very early age. Hammon (1949), for instance, working on the Pacific island of Guam, was able to demonstrate the presence of neutralizing antibodies in infants during their first year of life, usually in the complete absence of apparent illness.

If we accept the view that the presence of such antibodies indicates previous contact with the virus of poliomyelitis we can hardly escape the corollary that mild atypical attacks, or a purely immunizing carrier infection, must be an exceedingly common event in this particular disease. This conclusion is borne out by the finding of the virus in the throat or faeces of a varying proportion of family contacts of cases of poliomyelitis, and of non-contacts during epidemic periods (see Kling *et al* 1912, Flexner *et al* 1913, Kling and Pettersson 1914, Flexner and Amoss 1919, Lépine *et al* 1939, Brown *et al* 1948, Howe 1949, Hammon 1949, Casey *et al* 1950). From the distribution of the virus in the child population of Chicago, Casey, Fishbein, Schabel and Smith (1950) estimated that most children had been infected at least once by their 4th birthday, and 75 per cent. twice by their 6th birthday.

Yellow fever is another disease in which latent infections appear to be very common. Serum protection tests in South America and West Africa have shown that in endemic areas, where clinical cases are infrequent and sporadic, a high

species of fish, in some bacteria, such as certain of the paratyphoid and dysentery bacilli and pneumococci, and in some varieties of maize. It is not present in the erythrocytes of any of these animals but is present in the red cells of the sheep, whose organs do not contain it. It is not found in other organisms such as the pig, ox, rabbit, goose, frog, eel, man, pigeon and rat. This peculiar distribution among species has not been explained.

Still other antigens which are common to, or closely related in, widely different species are those which confer organ specificity. Possibly the immunologically related caseins may be considered in this category but, further, antigens present in a given organ such as kidney are similar to those in the same organ of a different species. These have been studied in some detail by Witebsky.¹⁰ The so called heavy proteins, isolated from disintegrated normal tissue by ultracentrifugation and thought by some to represent mitochondria, have been of interest in connection with the isolation of similar material from virus infected tissues which is believed to represent the infectious agent. Immunological studies on these substances by Furth and Kabat,¹¹ and by Henle, Chambers and Groupe,¹² have indicated that heavy proteins from various organs show three kinds of immunological specificity, namely, species specificity or immunological relation to other organs of the same animal, organ specificity, or immunological relation to the same organ of other species, and, finally, specificity for the organ of the one species. Similarly, Bailey and his co-workers¹³ have reported finding organ specificity in nutrient media which was derived from the tissues used for the preparation of the infusion. It has been reported by some that organ-specific antisera are specifically toxic for that organ, i.e., nephrotoxic, etc., but the evidence is not altogether unequivocal.

A number of antigens are common to different species of bacteria or to bacteria and certain other organisms and will be discussed in the following chapter in connection with natural immunity.

The Chemical Basis of Specificity.¹⁴ A large body of sound experimental evidence has established the fact that the specificity of antigens is determined by their chemical composition. Early experimental investigation of a wide variety of antigenic proteins has shown that immunologically identical proteins are, so far as can be determined, identical in composition; that antigenic proteins differing from one another in composition are also immunologically distinct, and that antigens showing some degree of cross reaction are closely related in chemical structure. Conclusive evidence that immunological specificity is a property of certain atomic and molecular arrangements has, however, been obtained through the study of altered specificity and artificial antigens.

The specificity of an antigenic protein may be altered by heating, partial denaturation, treatment with formaldehyde, etc., in such a way that part of the original specificity is lost but species specificity remains although somewhat

¹⁰495.

¹¹and Raffel. Amer. Jour. Med., 1941, 73:617.

¹²H.

¹³This subject is discussed in detail by Wells (loc. cit.) and by Landsteiner. *The Specificity of Serological Reactions*. Charles C Thomas, Springfield, Ill. 1936.

to remain latent in the tissues unless the equilibrium is upset. *Group 2.* Immunity persists but the virus does not. Examples are smallpox, chicken-pox, measles, yellow fever, and the insect-borne encephalitides. *Group 3.* Neither infection nor immunity persists. Influenza, the common cold, and foot-and-mouth disease are typical instances. *Group 4.* Both infection and immunity persist indefinitely, as in the transmissible tumours. The varying part played by active and passive immunity in these different groups will become evident during the course of this chapter.

The Mechanism of Virus Infections.

The general process of tissue invasion in virus diseases does not appear to differ in any essential way from that of bacterial infections. There would seem, in many diseases at least, to be the same sequence of local proliferation, blood-stream invasion and secondary foci of infection, each phase varying in prominence according to the virulence of the virus and the susceptibility or resistance of the host.

Thus, Todd and White (1914) note that, when cattle are injected subcutaneously with a small dose of the virus of rinderpest, the blood usually remains non-infective for 72 hours, becoming infective coincidently with the onset of illness: and Andrews and others (Report 1931) report that the virus of foot-and-mouth disease does not appear in the blood of experimentally infected cattle until shortly before the commencement of the febrile reaction, though it may be present in high concentration some hours before the temperature begins to rise, or before vesicles appear. The virus then tends to persist in the blood throughout the febrile period, though the degree and persistence of blood-stream infection varies widely in different animals (see also Waldmann, Trautwein and Pyl 1931). In yellow fever, Hudson and Philip (1929) find that monkeys bitten by infected mosquitoes show the presence of virus in their blood 1 to 2 days after infection, and at about the same interval before the onset of fever.

In many diseases characterized by a skin eruption the infecting virus can be shown to be widely distributed throughout the body. Even in vaccinia, in which the obvious lesions are commonly restricted to the site of inoculation into the skin, a viraemia may be present (Ohtawara 1922, Rivers and Tillett 1923, Gildemeister and Heuer 1927), and, with highly potent strains, this may lead to a widespread eruption and to lesions in the internal organs (Douglas, Smith and Price 1929).

The careful studies of Fenner (1949a) on mouse-pox (ectromelia) and of Downie (1951) on smallpox reveal a picture not unlike that of experimental mouse-typhoid infection (see p 2125).

After gaining access to the body, the virus produces a small primary lesion at the site of lodgment, passes to the lymphatic nodes and thence to the blood stream, where it causes a primary viraemia. Progressive infection occurs of cells in the internal organs till, towards the end of the incubation period, the virus overflows into the blood stream producing a secondary viraemia. At the beginning of the clinical disease pocks appear on the skin, and antibody becomes detectable a few days later.

In diseases affecting the nervous system, such as poliomyelitis and encephalitis, it used to be thought that the virus remained strictly confined to the nervous tissues, gaining access to the central nervous system by way of the axis cylinders and diffusing by neural pathways through the brain and cord. Improved technical methods, however, have shown that, even in these diseases, a transient viraemia may occur. In the insect-borne encephalitides the virus appears to reach the nervous system from the blood stream, and in poliomyelitis it apparently enters the blood from its site of primary lodgment before the central nervous system is obviously affected (see Chapter 87). Experimental observations on poliomyelitis

methods and something of their nature is known. The polysaccharide haptens referred to above are of very considerable immunological importance in many, but not all, cases. The isolation of such substances is relatively simple, usually involving a primary alcohol precipitation from solution of the bacterial cell substance. The somatic antigens of the enteric bacilli, which appear to be identical with endotoxin in many cases, may be prepared by extraction of the intact cells with M/2 trichloroacetic acid in the cold,²³ by extraction of the intact cells with glycols such as diethylene glycol,²⁴ by fractionation of tryptic digests of the cells,²⁵ or by dissociation and extraction in 6 M urea.²⁶ These substances may be precipitated from the crude extract by alcohol and further purified. Though it has been indicated earlier that these substances are polysaccharide-lipid complexes, there is not complete agreement on this point. Preparations made by primary trichloroacetic acid extraction do appear to be of this nature but those isolated by glycol extraction are polysaccharide-lipid-polypeptide complexes. These may be dissociated and reconstituted in hot formamide solution and the polypeptide portion seems to be essential to antigenicity. Sharp separation of the immunological specificity of the flagellar antigens has not been possible by chemical means, but physical separation in which the flagella are broken off by shaking and separated from the cell proper by differential centrifugation has been accomplished. The flagellar antigen is a protein in nature. In the "protein" separated from the flagella

The immunological entities have not been separated in such preparations, however.

The number of antigens that can enter into this mosaic in a single species is unknown, some *Salmonella* species have been found to contain seven or eight separate antigens which differ from one another not only immunologically but also in their resistance to heat, alcohol and the like, and these and other bacteria will undoubtedly prove to be increasingly complex with further study. The number of antigens demonstrable by antigenic analysis (p 300) is, of course, only a minimum since a given antigen remains an entity only so long as possible components of it have not been found to occur separately. It is of some interest that antigens co-existing in the same cell may not be equally demonstrable. The species specificity observable in rough pneumococci, for example, is present in the smooth forms but is masked by the predominant type-specific antigens. Similar phenomena have been observed in a variety of bacterial species. Conversion to varying degrees of roughness may bring to light "new" antigens which, though presumably constantly present, have been masked by the presence of other antigenic substances. There is a tendency to interpret such findings as indicating the relative position of antigenic substances within the cell, the peeling off of "outer" antigenic layers and exposure of secondary and tertiary layers of antigen within the cell. While there is something to be said for this concept as a figurative mode of expression, it may be pointed out that, aside from the fact that in some bacteria specific antigen is

²³ Borvin and Mesrobian: *Rev. Immunol.*, 1935, 1:553.

²⁴ Morgan: *Biochem. Jour.*, 1937, 31:2003.

²⁵ Raistrick and Topley: *Brit. Jour. Exp. Path.*, 1934, 15:113.

²⁶ Walker: *Biochem. Jour.*, 1940, 34:325.

to suggest that endotoxins, similar to those contained in certain bacteria, may be produced by viruses. Rake and Jones (1944), for example, found that the lymphogranuloma, meningopneumonitis, and mouse pneumonitis viruses (see Chapter 85), when grown in the yolk sac of the developing chick embryo, elaborated a thermolabile endotoxin capable on intravenous inoculation into mice of causing death in 4 to 24 hours (see also Manire and Meyer 1950). A similar toxic effect on mice, independent of multiplication of the virus, was observed by Henle and Henle (1916) after intracerebral inoculation of the virus of influenza. The development of tissue-culture techniques has shown that many viruses have a cytopathogenic effect, causing degeneration and death of the cells in which they are multiplying. How far this is due to toxic action and how far to interference with the metabolism of the cells by competitive growth is not yet clear. In Burnet's (1950) view the symptoms exhibited by patients suffering from virus diseases are due not to toxins but to soluble products derived from cell damage.

In one instance, at least, namely the action of the influenza virus on the red cell, the so-called toxin can almost certainly be identified with an enzyme. The phenomenon of *haemagglutination* is described in Chapter 74 but, briefly, it is found that the influenza virus is able to agglutinate the red blood corpuscles of fowls and certain animals. It probably does this by becoming adsorbed on to the cells and forming a bridge between them. If the virus-cell mixture is kept at 37° C for a time, the virus, which is at first removed by the cells from the fluid, sooner or later begins to reappear. Simultaneously the cells cease to be agglutinated, and remain inagglutinable even by fresh virus, though the eluted virus itself is able to agglutinate fresh red cells. The experimental evidence suggests that the virus secretes an enzyme of the mucinase class which acts on a mucopolysaccharide receptor in the cell. Much the same process occurs in the lung, except that, instead of being released from the cell, the virus enters and multiplies within it (see Hirst 1943, Burnet 1950). The *haemagglutinating* effect of the influenza virus can be neutralized non-specifically by certain lipid substances present in normal serum as well as specifically by influenzal antiserum. Numerous other viruses are known to cause *haemagglutination* under appropriate conditions, though the mechanism by which they produce this effect is not always the same. In vaccinia, for example, the *haemagglutinin* is not the virus itself, but a soluble product of it, containing a phospholipid component which is responsible for union with the surface of the red cell and which is destroyed by the *lecithinase* of *Cl. welchii* Type A—an enzyme that has no effect on *haemagglutination* by the influenza virus (Burnet 1946).

Besides a *haemagglutinating* action, a few viruses such as the virus of mumps and of Newcastle disease of fowls, are able to lyse the red cells of certain animals. Like the *haemagglutinin*, the *haemolysin* can be adsorbed on to red blood corpuscles and its action neutralized by specific antiserum, though the two enzymes differ in their heat stability and in other ways (see Morgan *et al* 1948, Chu and Morgan 1950).

Antiviral Immunity.

Before discussing the mechanism of antiviral immunity it will be convenient to consider briefly the ways in which such immunity may be induced; since it is held by many that, in this respect, there is a significant difference between the behaviour of the filtrable viruses and bacterial cells.

Active Antiviral Immunity.—We have seen that active immunity is conferred

present in the capsule or flagella, there is no evidence of an immunological geography in the bacterial cell.

The demonstration of the occurrence of common antigens in heterologous bacteria may be interpreted in two ways. Thus it may be assumed that the identical antigen, or at least its determinant portion, is shared. This is a common view and one for which there is a good deal of justification in many instances. It is believed by some, however, that the observed cross reactions are attributable to the occurrence of similar determinant groups and the type of partial antigen-antibody reaction observed with artificial antigens. As yet there is no definitive evidence to substantiate either view.

ANTIBODIES

The immunological response of an animal to the initial injection of an antigenic substance is not immediate but after a suitable time interval or incubation period is manifested as an alteration in the properties of the blood serum with respect to the antigen. An immune serum, or antiserum, differs from normal serum in that it reacts, either *in vivo* or *in vitro*, with the homologous antigen. This property of immune serum is a consequence of the presence of *antibodies*, substances which are formed by the animal body in response to the presence of antigen in the tissues, and which combine specifically with the antigen. The antigen-antibody reaction is demonstrable in a number of ways, the particular technique employed depending upon the nature of the antigen. By such means five apparently different kinds of antibodies may be found, although, as will appear, these are probably but a single substance. They are as follows:

- (1) the *antitoxins*—antibodies formed in response to the injection of toxins which, when mixed with the homologous toxin, neutralize its poisonous qualities,
- (2) the *lysins*—antibodies which bring about a dissolution or lysis of bacterial and other cells;
- (3) the *opsonins*—antibodies which sensitize bacterial cells in such a way that they are readily engulfed by the phagocytic cells,
- (4) the *agglutinins*—antibodies formed in response to the injection of bacterial cell substance which, when mixed with the homologous microorganism, immobilize the bacteria if they are motile, then aggregate, or agglutinate, the cells with the formation of clumps which settle out of suspension;
- (5) the *precipitins*—antibodies formed in response to the injection of antigens which, when mixed with soluble antigen, aggregate the molecules with the formation of a precipitate.

In addition to these five generally recognized kinds of antibodies, two others may be provisionally added:

- (6) *ablastins*—reproduction inhibiting antibodies which prevent the multiplication (cell division) of the invading microorganism.
- (7) *neutralizing antibodies*—antibodies which, when mixed and incubated with the infectious agent, generally a filterable virus, render it non-infective.

The various antibodies may be considered briefly one by one.

(d) By means of inactivated viruses. Numerous methods are employed for the process of inactivation. Drying was used by Pasteur in the preparation of his rabbit-cord virus against rabies (incidentally this method, like many others under this heading, is a combination of *c* and *d*); formalization by Curasson and Delpy (1926) and Daubney (1928) for cattle plague, by Laidlaw and Dunkin (1928*a, b*) for distemper, and in combination with heat for foot-and-mouth disease by Waldmann and his colleagues (1941-2); phenolization by Semple (1912) for rabies and by Todd (1928*b*) for fowl plague, crystal violet for swine fever (see Doyle and Wright 1917); and ultraviolet irradiation for rabies, St. Louis encephalitis and other virus diseases (Levinson *et al.* 1945). Sometimes the immunizing effect is increased by adsorbing the inactivated virus on to alum, as in Waldmann's vaccine against foot-and-mouth disease (Waldmann *et al.* 1941-2); sometimes an adjuvant is added, such as the mineral oil mixture used by Salk, Bailey and Laurent (1952) for influenza vaccine.

The degree of protection afforded by inactivated vaccines varies considerably, and it is sometimes necessary or desirable to reinforce their immunizing action by subsequent injections of living virus. The process of inactivation is not always easy to control, and with many vaccines a point is soon reached when not only the virulence but the antigenic potency of the virus is destroyed. If the processing is insufficient, then living active virus may survive, as happened with Kolmer's ricinoleate vaccine against poliomyelitis (see Chapter 87): if the processing is too thorough, then the vaccine may be practically devoid of immunizing power.

Whether an inactivated virus is a dead virus is very difficult to decide. A great deal of ambiguous evidence might be analysed without enabling us to arrive at a firm conclusion. Moreover, since the same agent in the same concentration may vary in its effect on two different viruses, it would be impossible to discuss this subject satisfactorily without reviewing the studies on a great number of viruses inactivated in different ways.

W. Henle and G. Henle (1947) found that the various activities of the influenza virus were affected at different rates by ultraviolet irradiation. The properties were affected in the following order: (1) ability to propagate, (2) toxic action as tested by intravenous inoculation of white mice, (3) ability to elicit the interference phenomenon (see p. 1430) and to inhibit the development of the chick embryo; (4) haemagglutinating capacity; and (5) complement-fixing activity. The immunizing power began to decrease before the haemagglutinating power fell, but was still appreciable after all the haemagglutinating power had been lost.

There is plenty of evidence to show that many inactivated vaccines in which no living virus can be demonstrated by any available means are good immunizing agents, and this is really as far as we can go. To attempt to pass beyond this is to enter the realm of metaphysical speculation and to face the difficulty of defining the meaning of live and dead in relation to small particles of biologically active material (see Chapter 41).

Passive Antiviral Immunity.—Turning to passive immunity, enough has already been said to indicate that antiviral sera are at least as effective in affording protection against the homologous virus as is an antibacterial serum in protecting against the homologous bacterium. It may be noted that, though antibacterial sera are commonly prepared by the immunization of some conveniently large animal, usually the horse it has become the practice to prepare antiviral sera by the

Antitoxins. It was found by von Behring and Kitasato²⁷ in 1890 that the immunity of rabbits and mice which had been immunized against tetanus was associated with the ability of the blood serum to neutralize the toxic substances produced by the tetanus bacillus. The substance in the serum which neutralized the tetanus toxin was designated by these workers as *antitoxin*. Subsequent investigation has shown that the animal body forms antitoxins in response to the injection of a variety of antigenic poisons, not only those of bacterial origin such as diphtheria toxin, botulinus toxin and the like, but also against the phytotoxins and zootoxins (p. 206).

The action of antitoxin may be directly demonstrated in the following way: if a fatal, or many times fatal, dose of toxin be mixed with an appropriate amount of antitoxic serum *in vitro*, the injection of the mixture into a susceptible animal is wholly without injurious effect; the poisonous qualities of the toxin are nullified by the immune serum. The reaction is, like all other immunological reactions, highly specific, and an antitoxin which neutralizes the homologous toxin is without effect on heterologous toxins. The nature of the effect of the antitoxin which renders a powerful toxin pharmacologically inert is unknown.

The Toxin-Antitoxin Reaction. The combination of toxin and antitoxin does not necessitate the complete destruction of either component; neutral mixtures of toxin and antitoxin may be dissociated by treatment with hydrochloric acid, by freezing in the presence of phenol or tricesol and, to some extent, by simple dilution. In certain cases, *e.g.*, pyocyaneus toxin and certain snake venoms, in which the toxin is more resistant to heat than the antitoxin, the latter may be selectively destroyed and the neutral mixture becomes toxic upon judicious heating. It appears, therefore, that a more or less loose combination of toxin and antitoxin takes place, the poisonous properties of the toxin being held in abeyance as long as the union persists. The rate of reaction between toxin and antitoxin, like the chemical reactions, is dependent upon temperature, concentration, character of the medium in which the reaction occurs, and similar factors. The avidity of an antitoxin for its corresponding toxin differs in different cases; the union between tetanus toxin and antitoxin, for example, takes place less rapidly than that between diphtheria toxin and antitoxin.

An understanding of the precise character of the toxin-antitoxin reaction is dependent upon the interpretation of phenomena revealed by quantitative studies. It might be expected that a given quantity of antitoxin would always neutralize a constant amount of toxin, that the neutralization would follow the law of multiple proportions. This is, however, not the case, and it appears that the amount of antitoxin required to neutralize a given quantity of toxin is dependent upon (a) the manner in which the two are mixed with one another and (b) the relation between toxicity and combining power in the particular filtrate under consideration.

✓ In the first instance it has been observed that when an excess of toxin is added to its specific antitoxin in several portions at proper intervals of time, much more unneutralized toxin remains in the mixture than if the same quantity of toxin had been added to the same quantity of antitoxin at one time. This is known as the *Danysz phenomenon*. If, on the other hand, antitoxin be

²⁷ von Behring and Kitasato. Deut. med. Wchnschr., 1890, 16 1113.

From the work of Salaman (1937) it seems clear that the neutralizing antibody to vaccinia becomes fixed to the virus particles themselves. Absorption with elementary bodies, but not with the soluble specific substance that takes part in the complement-fixation and precipitation tests, removed the virus-neutralizing bodies from a serum. The observations recorded above suggest that the neutralizing antibody does not lead directly to the death of the virus; and the finding of virus and antibody together in the circulating blood or in growing tissue cultures in vaccinia (Smith 1929), ectromelia (Downie and McGaughey 1935), and psittacosis (Bedson 1937) is additional evidence in favour of this view. The experiments of Andrewes (1929*a, b*) on Virus III and of Rivers, Haagen and Muckenfuss (1929*a, b*) on vaccinia and herpes show that in tissue cultures the formation of characteristic inclusion bodies can be prevented by the addition of immune serum to the cultures before the virus. This may be interpreted as meaning that the antibody interferes with the penetration of the cell by the virus, or that it so alters the virus as to prevent its multiplication when it reaches the interior of the cell.

As with the antibodies acting on bacteria and their products, the antibodies in antiviral sera appear to be confined to the globulin fractions. The early observations of Hartley (1914) on antiserum to cattle plague, of Henseval (1919) and Ledingham, Morgan and Petrie (1931) on vaccinal antiserum, of Matland and Burbury (1927) on antiserum to foot-and-mouth disease, of Weyer, Park and Banzhaf (1929) and Morgan and Fairbrother (1930) on poliomyelitis antiserum, and of Laidlaw and Dunkin (1931) on distemper antiserum—all pointed to the close association of antibody with the serum globulin. There was some disagreement on the particular fraction of the globulin—*α*-globulin or pseudoglobulin—which was most active; but the more recent work of Cohn in the United States of America and of Kekwick in this country leaves little doubt that the neutralizing antibody is concentrated mainly in the gamma globulin fraction (see Chapter 88).

Non-specific Inactivating Agents.—Working with influenza virus Burnet and McCrea (1916) observed that normal ferret sera had quite a strong inhibitory action on hæmagglutination and on the development of the virus both in eggs and in mice. Ginsberg and Horsfall (1949*b*), like numerous other workers, extended this observation to the sera of man, rabbits, guinea-pigs and mice, and showed that the heat-labile component of the serum which was responsible for inhibiting hæmagglutination also neutralized the infectivity not only of the influenza virus but of the Newcastle disease and the mumps viruses as well. Though the component was destroyed by heating to 56° C., there was no suggestion that it was the same as complement, because it did not act in conjunction with antibody, as complement does. It seems probable that the inhibitory component is of lipid nature.

Francis (1947*b*) showed that many apparently normal sera contain a substance which inhibits hæmagglutination by killed influenza B virus but not by living virus. Thus the apparent antibody titre of a serum may be much higher against killed than against living virus—an effect referred to as the *Francis phenomenon* (see Chapter 74).

Another non-specific inactivating agent, of more limited scope, is found in certain polysaccharides. Green and Woolley (1947) noticed that apple pectin, gum acacia, and flaxseed mucilage inhibited the agglutination of chicken red cells by influenza A virus, and that apple pectin interfered with the multiplication of the virus in fertile eggs. Ginsberg, Goebel and Horsfall (1948) showed that the polysaccharide extracted from Friedlander's bacillus inhibited the growth of mumps virus in the allantoic sac of the chick embryo. As little as 5 µgm. was effective. The growth

added to toxin in successive equal portions, it may be shown that, in general, the first portion of antitoxin neutralizes a greater portion of the toxin than the second, the second a greater than the third, etc. In the second case it has been found that there is no constant relationship between toxicity and combining power of a toxic filtrate; toxicity slowly diminishes upon storage but

Such altered toxin, which retains its antigenicity unchanged in addition to its combining powers, is designated as *toxoid* and, when prepared by treatment with formaldehyde, *formol toxoid* or *anatoxin*. The proportions of toxin and toxoid in a given filtrate are variable. Consequently there is no fixed relation between toxicity and power to combine with antitoxin; the MLD's neutralized by a unit quantity of antitoxin may vary from one filtrate to another or in the same filtrate at different times from 30 to 130.

Three theoretical explanations of the toxin-antitoxin reaction have been advanced. They are known by the names of their proponents, Ehrlich Arrhenius and Madsen, and Bordet and Landsteiner.

According to Ehrlich the reaction is purely chemical and essentially similar to the neutralization of a strong acid by a strong base. The combining properties of toxin and antitoxin would, therefore, be a manifestation of primary valencies. This concept is, however, not compatible with the observed behavior of toxin and antitoxin in mixture, the Danysz phenomenon, for instance, suggests that if the neutralization proceeds according to Ehrlich, the reacting substances are not homogeneous but consist of mixtures of substances with varying affinities for one another. On the basis of his studies on diphtheria toxin, Ehrlich postulated a series of components of toxin which differed from one another both in their avidity for antitoxin and in their toxicity.

Arrhenius and Madsen preferred to regard the toxin-antitoxin combination as essentially weak acid by a weak

is attractive in a toxin-antitoxin reaction. Simple dilution of a neutral mixture, for example, does not result in the degree of dissociation that this theory calls for. As a consequence of this and certain other discrepancies, the theory of Arrhenius and Madsen, is, like the theory of Ehrlich, not generally accepted at the present time.

A third concept, proposed by Bordet and strongly supported by Landsteiner, is that of the toxin-antitoxin reaction as an *adsorption phenomenon*, essentially physico-chemical in nature and arising as a consequence of intermolecular forces (secondary valencies). The varying degrees of toxicity apparent in the neutralization of toxin may be regarded as due to differences in completion of saturation of the individual toxic units, a process that may be compared to certain staining reactions such as the action of iodine upon starch, a dilute iodine solution producing a light blue tinge, a stronger solution a deep blue. This adsorption theory would view the action of antitoxin upon toxin as a sort of progressive attenuation, proportional to the amount of antitoxin added. The evidence for such a mechanism, not only in the toxin-

ovaries among the females. Thus we find the highest frequencies in those tissues for which vaccinia virus is known to have a special affinity and in those organs—lungs, liver, spleen and adrenals—which are primarily concerned in freeing the blood stream from inanimate foreign particles or from bacteria.

According to Flexner and Amoss (1914), the injection of poliomyelitis virus into the veins of monkeys—a route that does not commonly lead to the production of the typical disease—is followed by the prompt deposition of the virus in the spleen and bone marrow, but not in the kidneys. Galloway (Report 1931) recorded a few experiments in which the infectivity of various organs was determined 24–48 hours after the injection of foot-and-mouth virus into the pad of the guinea-pig's foot. The concentration of virus was highest in the blood; the spleen, liver and lungs contained virus in detectable amount, though the mesenteric glands, ovary, testis and muscles did not. In dog distemper (Laidlaw and Dunkin 1928*a, b*), organs rich in reticulo-endothelial cells, such as the spleen, show a high virus content. Valuable observations on the distribution of the mouse-pox virus in the tissues of experimentally infected animals at different stages of the disease were made by Fenner (1949*a*), whose series of papers will well repay study.

A review of the available data appears to justify the following conclusions. Where the virus concerned has an affinity for some special tissue in which it causes its characteristic lesions, it will, naturally enough, be found in the greatest concentration in those lesions or in their immediate neighbourhood. Thus we find vaccinia virus in the highest concentration in the lymph of the pustules, the virus of foot-and-mouth disease in the fluid of the vesicles, the virus of rabies, or of poliomyelitis, or of Borna disease, in the central nervous system, and so on; but even in these diseases the other tissues in which the virus is most frequently present, if we except the blood, are those concerned in the normal clearing mechanism—the spleen, liver, lungs, bone marrow or adrenals. In generalized infections, in which localization in the skin or nervous system is not a feature of the disease, the reticulo-endothelial tissues frequently show the highest virus content.

The blood we must consider in rather more detail. That the virus may often be demonstrated in blood withdrawn during the febrile stage of illness, or even at other times, we have already seen; but many of the results recorded, particularly in the later stages of experimental infections, have been curiously irregular. It would appear that these irregularities have been due in part to the fact that the virus is often present in the cellular elements—particularly in the leucocytes—rather than in the plasma, and that viricidal antibodies may appear in the plasma, while virus is present in the cells.

As long ago as 1899 Kolle showed that the infective agent in the citrated blood of an animal suffering from cattle plague was readily removed by centrifugation. The supernatant plasma was non-infective; the deposit, containing the blood cells, infective to a high degree. Todd and White (1914) studied this phenomenon in greater detail and showed that the virus was mainly associated with the leucocytes, and Schein (1917) and Daubney (1928) recorded similar findings. Russ (1906) (see also Landsteiner and Russ 1906) found that the virus of fowl plague was present in greater amount in the cellular constituents of the blood than in the plasma, and Todd (1928*a*) showed that the concentration was highest in the leucocytic layer, though Doerr and Gold (1932) recorded experiments which they interpreted as indicating an adsorption of the virus by the red cells.

Smith (1929) made a careful study of this problem in experimental vaccinia in the rabbit. Comparing the infectivity of the whole blood, the plasma, and the washed deposit of cells during the early days of a generalized infection he found that the washed cells were most infective, and the plasma non-infective. By fractional centrifugation it was possible to show that the red cells played no part in fixing the virus. The results suggested

antitoxin reaction but in antigen-antibody reactions in general, is very strong indeed, and it is generally regarded as highly probable that these reactions are essentially physico-chemical in nature. This view of the immunological reactions will be considered at greater length in a later section.

The Standardization of Antitoxins. The quantitative evaluation of the toxin-neutralizing capacity of antitoxic sera is clearly a matter of considerable practical as well as theoretical importance. Ehrlich originally proposed as the standard unit of diphtheria antitoxin that amount which would just neutralize 100 guinea pig MLD's of toxin. As indicated above, however, variability in the relation of toxicity to combining power invalidates any standard based on toxicity; in other words, the combining power of a toxin is not a measure of its toxic qualities. On the other hand, since the combining power of a toxic filtrate remains constant within narrow limits, it is possible to establish an arbitrary standard unit upon which the relative strength of all antitoxic sera can be based. Such a standard diphtheria antitoxin was first prepared by Ehrlich and was preserved by him with all precautions against possible deterioration. A standard antitoxic serum based on Ehrlich's arbitrary standard unit is also prepared in this country by the National Institute of Health of the United States Public Health Service, and is distributed every two months to the licensed producers of commercial serum. The unit is international, standard sera being tested from time to time by the Biological Standardization Commission of the League of Nations.²⁸

Three methods are used in the titration of diphtheria toxin and antitoxin. The first of these is the classic method of Ehrlich in which two limits (Lat., *limes*) are determined by guinea pig inoculation. These are the L_0 dose of toxin, which is defined as the standard unit of antitoxin, and the L_+ when mixed with one unit of standard antitoxin. The L_0 dose of toxin is the amount which produces a 100 per cent mortality in the inoculated guinea pigs is considered to contain one unit of diphtheria antitoxin.

A second method is based upon the observation that the intradermal injection of 1/250 to 1/500 MLD of diphtheria toxin into a guinea pig is followed by a local reaction, swelling and erythema and, with slightly larger amounts of toxin, necrosis, a phenomenon sometimes called the *Romer reaction*. By the use of such intradermal inoculations an L_r dose of toxin may be determined, i.e., that amount of toxin which, when mixed with one standard unit of antitoxin, will produce the minimal skin reaction. The serum to be standardized is mixed in varying amounts with L_r doses of toxin, and that amount of serum which gives the minimal skin reaction is considered to contain one unit of antitoxin.²⁹ This method has the advantage of allowing the testing of a number of toxin-antitoxin mixtures in one animal but has not displaced the classic method in common usage.

The third method makes use of the Ramon flocculation, the *in vitro* precipita-

²⁸ Cf. Amer. Jour. Public Health, 1935, 25:712.

²⁹ Glenny and Allen: Jour. Path. and Bact., 1921, 24:61

be understood that, when we use the term "cells" in relation to viruses, we are generally referring not to the reticulo-endothelial cells of the body, which are so active in antibacterial immunity, but to the particular cells invaded by the virus. The reticulo-endothelial system, as we have just seen, undoubtedly acts in conjunction with neutralizing antibodies to dispose of certain types of virus; but when we speak of cellular immunity in virus diseases we have in mind an entirely different mechanism, namely the resistance of normally susceptible cells to invasion by active virus particles. We know that neutralizing antibodies, if mixed with virus before inoculation, or if injected into an animal before the virus, are capable under suitable conditions of preventing infection. Even, however, in virus infections in which a strong neutralizing antibody can be obtained the available evidence suggests that inactivation of the virus can occur only extracellularly. Once the virus has penetrated the cell, it seems to be adequately shielded from the antibody. When, as in the prophylaxis of measles, the antibody reaches the majority of the susceptible cells before the virus, the virus may be neutralized either on the surface of the cell or in its immediate vicinity; but if the serum is given too late, after the virus has penetrated the cells, it is almost or completely without effect. Again in smallpox, as Downie (1951) showed, neutralizing antibody developing within a few days of the onset of the disease has no power to prevent the maturation of the skin lesions, because the virus is already protected by virtue of its intracellular position. In fact, patients may die in the pustular stage of the disease having a high titre of antibody in the serum, in much the same way as a horse used for the production of antiserum against tetanus will die if the toxin is injected directly into the brain where it is inaccessible to the serum antibody (For further studies on the protective effect of the cell on viruses, see Perdrau and Todd 1936)

Though neutralizing antibodies, if appearing naturally during the course of the disease or given artificially after the cells have become infected, may be unable to hinder the development of the lesions, there is good reason to believe that the degree of immunity after recovery is closely related to the persistence of these antibodies. As Beveridge (1952) points out, the diseases belonging to Francis' Group 2 (see p. 1415), such as smallpox, yellow fever and the insect-borne encephalitides, are systemic diseases characterized by the development of neutralizing antibodies which persist in the circulation, often for years. Another feature of this group of diseases is that they are caused by only one antigenic type of virus. Francis' Group 3, on the other hand, comprises diseases such as (a) influenza, poliomyelitis and foot-and-mouth disease, in which there is more than one antigenic type of virus, so that neutralizing antibodies developed against one type do not protect against infection with a different type, and (b) herpes febrilis in which the lesions are restricted largely to the surface of a mucous membrane where there is little neutralizing antibody available. In neither of these groups is there any need for predicated the existence of a specific cellular immunity to explain the freedom or otherwise from second attacks.

What about Francis' Group 1, however, of which psittacosis and lymphogranuloma are the outstanding examples? In these diseases the virus often persists in the tissues for a long time, recrudescences may occur at intervals, and no lasting immunity develops. It is significant that in this group neutralizing antibodies, except perhaps in very low titre, cannot be demonstrated. Why this should be so, it is impossible to say, but it may be remarked that these viruses are among

tion of toxin and antitoxin when mixed in optimal proportions.³⁰ Standard antitoxin is mixed with varying quantities of toxin, and the tube first, i.e., in time, showing precipitation contains one Lf dose of toxin. Varying amounts of the serum to be standardized are mixed with the Lf dose of toxin. The amount of serum in the tube first showing flocculation in this second series is considered to contain one unit of antitoxin. This method differs from the other two in that it depends upon the combining power of a toxic filtrate rather than on toxicity. It is generally used, not as a final method of standardization, but as a preliminary to standardization by the Ehrlich method.

The interrelationships of these limits are of some interest. The L+ dose is, of course, larger than the L₀ dose, and, because of the peculiarities of the toxin-antitoxin reaction, by considerably more than one MLD. The Lr dose approximates the L₀ dose as might be expected in view of the small amount of toxin required to elicit the skin reaction. The Lf dose is generally somewhat less than any of these, since it is a measure of combining power rather than toxicity and is unaffected by differences in the proportions of toxin and toxoid. It would appear that the Lf/Lr ratio should be the same for a given toxic filtrate immediately upon standardization. It has been found, however, that this ratio differs with different antitoxic sera. This appears to be a consequence of differences in avidity, not of hypothetical toxin components as suggested by Ehrlich, but of antibody. The avidity of antitoxic sera is associated with the protein fraction containing the antibody; successive globulin fractions differ from one another in avidity (see also p. 314).

The Bactericidal Substances—Lysins. It was early shown by Nuttall³¹ that fresh, defibrinated animal blood is markedly bactericidal. This property, also possessed by cell-free serum, was found to be heat-labile and destroyed by holding at a temperature of 55° to 56° C. for thirty minutes. Such heated sera are said to be *inactivated*. The blood or serum from a single animal species is not equally active on all species of bacteria and, conversely, a given bacterial species is affected to varying degrees by the blood of different animal species. It was suggested by Buchner that the natural resistance of an animal to infection could be explained in part by this bactericidal quality of the blood, and he proposed the name *alexin* (Gr., to ward off) for the heat-labile activity.

Although this bactericidal activity of the blood and serum of normal animals is frequently specifically increased by immunization, such an increase does not invariably accompany the development of the immune state. The serum of guinea pigs immunized with *Vibrio metchnikovii* is strongly germicidal for that microorganism, while that of unimmunized guinea pigs is devoid of specific bactericidal quality. On the other hand, immunization with streptococci, while producing a specific resistance to infection with the bacterium, frequently fails to induce an increased, specific germicidal activity.

Pfeiffer Phenomenon. The bactericidal effect is, in some instances, accompanied by visible dissolution of the bacterial cells. The course of events following the introduction of cholera vibrios into the peritoneal cavity of an immunized animal was followed microscopically by Pfeiffer³² and described by

³⁰ Ramon. *Compt. Rend. Soc. Biol.*, 1922, 86 661, 711, 813.

³¹ Nuttall. *Ztschr. f. Hyg.*, 1888, 4 353.

³² Pfeiffer and Isacoff. *Ztschr. f. Hyg.*, 1894, 17 355.

antibody response to a non-specific stimulus was recorded by Horsfall and Curnen (1946) in mice latently infected with the pneumonitis virus and injected intranasally with a suspension of normal chick embryo.

Interference Phenomenon.—Though most of the phenomena of immunity in virus diseases can be explained on the basis of humoral antibodies, experimental observations in the laboratory, made first by Hoskins (1935) and later by Findlay and MacCallum (1937) and a host of other workers, have shown that a form of cellular resistance may be induced by certain special procedures. It was found that monkeys inoculated with a pantropic strain of yellow fever virus died; but that if neurotropic virus, which is not normally fatal, was injected at the same time, the animals survived. Since the precocious development of antibodies seemed to be ruled out, the explanation offered was that the neurotropic variant occupied a proportion of the cells that would normally have been invaded by the pantropic virus and so prevented them from being infected by this fatal form of the virus.

Numerous other examples of this interference phenomenon, as it is now called, might be quoted. References will be found in the review by Henle (1950). In some instances the phenomenon can be reproduced by an attenuated virus, as in the protection of dogs against distemper by the injection of a distemperoid virus (Green and Stulberg 1946); sometimes by another virus of the same group, as in the inhibition of growth of the psittacosis virus in the fertile egg by previous injection of the virus of meningo-pneumonitis (Golub and Wagner 1948); sometimes by another virus of a different group, as in the ability of a non-neurotropic influenza virus injected intracerebrally to protect mice against subsequent infection with the virus of Western equine encephalomyelitis (Vilches and Hirst 1947); and sometimes by the same virus after inactivation (see Isaacs and Edney 1950). In some instances the protecting virus has to be injected into the same tissue as the test virus: in others the two viruses may be injected into different tissues. The interference phenomenon may likewise be called upon to explain the difficulty of demonstrating by infectivity tests small numbers of living virus particles in the presence of large quantities of dead virus (Andrewes and Elford 1947).

Immunity of this type, in which a cell already occupied by a virus is resistant to infection by other particles of the same or nearly related virus, may perhaps be referred to as *pre-emptive immunity*—a term suggested by Dr. C. H. Andrewes. We know that such an immunity occurs in plants and in the developing chick embryo, in which humoral antibodies have never yet been demonstrated; and there is increasing evidence that it plays a part in the defence of the host against some of the animal virus infections. It must be made clear, however, that the phenomenon occurs only under special conditions and that it is by no means universal.

Syvertson and Berry (1947), for example, working with virus-induced tumours, were able to infect cells with two viruses at the same time. Thus the cornea of the rabbit could be infected simultaneously with vaccinia and with herpes virus, as shown by the presence of cytoplasmic and intranuclear inclusion bodies in the same cells. Incidentally, as the authors point out, the isolation of a given virus from a tissue or tumour does not necessarily prove that this virus was responsible for the lesions observed.

The mechanism of the interference phenomenon in virus infection is clearly independent of any specific response of the host cell to the antigens of the blocking virus, since the blocking and the blocked virus are in many instances antigenically

him in detail. The vibrios first lose their motility, then swell up and crumble into small fragments. The dissolution of the fragments follows, and no trace of the bacterial cell remains visible. This action of the body fluids is attributable to the presence of an antibody termed a *lysin* (Gr., to loose, dissolve). It occurs not only within the peritoneal cavity of an immunized animal, but also when the peritoneal fluid or blood serum is removed from the body and brought immediately in contact with the bacteria *in vitro*. The lytic activity of bactericidal sera *in vitro* is heat-labile and the process of lysis appears to be essentially identical with that observed *in vivo*.

Visible lysis of bacterial cells, however, does not invariably accompany the lethal activity of a bactericidal serum. In fact, the majority of bacteria are not dissolved by immune sera in the manner described above. This is not to be taken to indicate that the processes are fundamentally different; not only are many bacteria highly resistant to visible structural alterations, viz., the lack of sensitivity to wide variations in osmotic pressure, mechanical pressure, etc., but, as will appear, the antigen-antibody reaction takes place in two stages, the first the union of antigen and antibody and the second the visible consequences of that union. Whether or not dissolution of the bacterial cells occurs, it may be readily shown that they unite with the lysins.

Hemolysis. The lysis of bacteria by an immune serum is not a unique reaction in which only bacterial cells may play a part; bacteriolysis is, rather, a special case of a general phenomenon, for immunization with a variety of cells results in the production of cytolytic sera. Of these, erythrocytes have been by far the most widely studied, for the lysis of these cells, hemolysis, is readily apparent in the test tube; the red opacity of the cell suspension changes to the clear red solution of hemoglobin as lysis proceeds. The stroma is not dissolved but, upon examination, appears misshapen. First demonstrated by Bordet,³³ hemolysis has been particularly useful not only as a type of lytic reaction peculiarly suitable for laboratory manipulation, but also as an indicator of antigen-antibody combination when visible lysis does not or cannot take place. It may be noted that the immune hemolysins, i.e., those formed by the animal body in response to the injection of erythrocytes, are to be distinguished from the filterable hemolysins formed by bacteria (p. 206).

Antisera have been prepared against a variety of other cells. The injection of spermatozoa leads to the appearance, in the serum of the inoculated animal, of a spermatotoxic substance that first renders the corresponding spermatozoa motionless and then kills them. A number of similar cytolytic sera have been prepared—"nephrotoxic" sera by the injection of kidney cells, "hepatotoxic" sera by the injection of liver cells, etc., but the organ specificity claimed for these last has not been satisfactorily demonstrated.

The Mechanism of the Lytic Reaction. An observation contributing in large measure to a partial understanding of the mechanism of the action of the immune lysin was that lytic or bactericidal activity could be completely restored to an inactivated serum by the addition of a small amount of fresh unheated serum, either normal or immune. It appears, therefore, that the

³³ Bordet: Ann Inst. Pasteur, 1898, 12:688.

after infection. It had no direct action on the two viruses *in vitro*, and did not affect their absorption by the host tissues; and it did not affect the influenza and Newcastle viruses. The presumption that it blocked a metabolic system in the host cell which was used only by the pneumonitis and mumps viruses was borne out by the demonstration of interference of the pneumonitis by the mumps virus and of no interference of influenza or Newcastle disease viruses by mumps or pneumonitis virus, and *vice versa*. The influenza and Newcastle disease viruses, which interfered reciprocally with one another, evidently depend in common on a single metabolic system in the host cells that is insusceptible to the Friedländer polysaccharide (Horsfall and McCarty 1947, Ginsberg *et al.* 1948, Ginsberg and Horsfall 1949a).

Closely allied to the interference phenomenon is what Beveridge (1950) called the *excessive dose effect*. Many instances can be quoted of virus diseases in which a small dose of living virus gives rise to a severer reaction than a large dose. For a discussion of the possible explanation of this effect, reference should be made to Beveridge's article.

Allergy in Virus Diseases.

The characteristic response to reinoculation with vaccinia virus affords one of the classical examples of an allergic reaction. The typical sequence of events after a successful primary inoculation commences with an incubation period lasting about 3 days, followed by the appearance of papules at the sites of inoculation on about the 4th day. These develop into compound vesicles during the next 5 days, become definitely pustular about the 10th day, and heal by the well-known scabbing process between the 14th and 21st days. If a person who has been successfully vaccinated is again injected with calf lymph during the period of waning immunity, but before he has again become fully susceptible, the most noticeable feature in the local reaction is an acceleration of all stages. The papules appear earlier, sometimes within 24 hours; and vesiculation and pustulation, when they occur, are in evidence at a far earlier period than after primary vaccination. Very frequently, however, the reaction ceases at the papular stage, and this may be so transitory as to be missed unless daily examinations are made. In any case, the induration round the papules or vesicles is usually far less marked after a secondary than after a primary vaccination, and the constitutional symptoms are slighter. We have, in fact, the typical allergic combination of accelerated response with localization of infection. It is known that in previously vaccinated persons the so-called immediate reaction, reaching its maximum within 72 hours, can be produced by heated vaccine as well as by live vaccine (see Andervont and Rosenau 1930, Broom 1947), whereas it does not occur in unvaccinated persons unless they have suffered from smallpox. There is reason, therefore, to regard it as an allergic response. An increased reactivity of the skin to injection with live or inactivated virus can be demonstrated in persons or animals that have recovered from a variety of other virus diseases, such as psittacosis, lymphogranuloma, influenza, mumps, and swine fever.

In general, the more recent the infection, the stronger is the degree of allergy. This fact leads to the question whether allergy is merely an indicator of immunity, or is actually an integral part of it. Without entering into a discussion of this topic here (see pp. 1331 and 1522), it may be remarked that Beveridge (1952) would regard the allergic condition as part and parcel of immunity. In his view

lytic reaction is a consequence of the interaction of three components, rather than two as in the case of the toxin-antitoxin reaction. One of these is clearly an immune body, *i.e.*, is formed in response to the injection of the antigen, and another, the heat-labile component present in normal as well as immune serum. The third component is, of course, the antigen.

The heat-labile component was termed alexin by Buchner,³¹ as indicated above, and renamed *complement* by Ehrlich. The immune body or antibody was called *substance sensibilisatrice* or *sensitizer* by Bordet and *amboceptor* by Ehrlich. The relation between these components may be expressed in tabular form for a hemolytic system

erythrocytes (antigen)	+	unheated immune serum (complement or alexin + amboceptor or sensi- tizer)	=	hemolysis		
erythrocytes (antigen)	+	heated immune serum (amboceptor or sensiti- zer)	=	no hemolysis		
erythrocytes (antigen)	+	heated immune serum (amboceptor or sensi- tizer)	+	unheated normal serum (complement or alexin)	=	hemolysis

These components react with one another, not at random but in an orderly manner; the union between antigen and amboceptor must precede the reaction with complement. Complement does not combine with antigen in the absence of amboceptor, but antigen and amboceptor will unite regardless of the presence of complement. This may be demonstrated directly by using mixtures of only two components or in the presence of all three by holding the mixture at 0° C.; the antigen-amboceptor union readily takes place at this temperature, but complement unites only slowly and may be demonstrated in the supernatant following centrifugation.

According to Ehrlich, complement acts upon the antigen only indirectly through the amboceptor, the last functioning as a bridge between the first two. In this connection it is of interest that colloidal silicic acid may act as a sensitizing agent in the lysis of red cells. The evidence does not require that the action of complement be indirect in this way; it is established only that the antigen must be sensitized by the antibody before union with complement can occur. According to Bordet the union is in the nature of a specific adsorption, the sensitized antigen being rendered susceptible to the lytic action of the complement. For example, it is well known that tannic acid will act as a sensitizer, and treated erythrocytes are lysed in the presence of complement. The evidence, which cannot be considered at length here, strongly supports this view. In consequence it is generally admitted that the terms alexin and sensitizer are preferable to complement and amboceptor, although the latter remain in common usage. Quantitative studies by Heidelberger and his co-workers³² have shown that under ordinary circumstances

³¹ Buchner originally used the term alexin to designate the entire bactericidal action of the blood serum, alexin was later used by Bordet for the heat labile substance alone; and at the present time alexin is regarded as synonymous with complement.

³² Heidelberger Jour. Exp. Med., 1941, 73-681. Heidelberger, Weil and Treffers, *ibid.*, 1941, 73-695. Heidelberger, Rocha e Silva and Mayer: *ibid.*, 1941, 74-359.

in deciding whether tonsillectomy predisposes to an attack of the disease or merely to the bulbar form of it, but doubtless suitable studies will show before long which is correct.

Numerous observations are on record to show that paralysis may follow shortly after certain prophylactic injections, particularly of vaccines such as combined diphtheria and pertussis vaccine alum precipitated. Paralysis usually develops within 3 weeks and affects first of all the injected limb. Here again, whether the vaccine predisposes to an attack of poliomyelitis or merely to the localization of paralysis at the site of injection is not clear, though the evidence points towards the former (see McCloskey 1950, Hill and Knowelden 1950).

The effect of nutrition on altering resistance to virus infection is very imperfectly understood. The general impression that infantile paralysis picks out the healthiest and best fed children may have nothing to do with nutrition as such, but may be associated with the hygienic conditions which tend to prevent the better housed social classes from suffering from latent or subclinical infections at an early age. The part played by various nutritional deficiencies was summarized in an article by Findlay (1948), which deals also with the effect of other non-specific factors on resistance to virus disease. (See also p. 1374.)

An interesting example of activation of the virus of mouse hepatitis by *Eperythrozoon muris* is recorded by Andrewes and his colleagues. So far none of the viruses with the exception of those of the lymphogranuloma-psittacosis group, have proved amenable to treatment with chemotherapeutic or antibiotic agents (see Hurst 1953).

Useful reviews, which have been freely drawn on in the writing of this chapter, have been published by Bedson (1937), Burnet, Keogh and Lush (1937), Andrewes (1939, 1952), Rivers (1943), Francis (1947a), Fenner (1949a), Hammon (1949), Downie (1951), Beveridge (1952), and Wilson Smith (1953).

SUMMARY

It is perhaps a fair summary of the evidence presented in this chapter to suggest that it is compatible with the view that there is no essential difference between the mechanisms concerned in antiviral and antibacterial immunity. It would appear that neutralizing antibodies play a greater part and phagocytosis a smaller part in the defence of the body against viruses than against bacteria. In so far as immunity to virus infections is more effective than immunity to bacterial invasion, the difference may be due rather to the greater limitations imposed on the virus by its habit of intracellular parasitism than to any special reaction on the part of the host.

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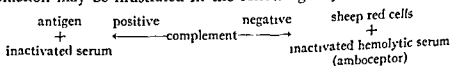
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about seven molecules of complement are required for each molecule of antibody, but in very dilute solution the ratio may approach unity. These workers believe that complement exists in a loose union with antibody but is tightly bound in the antigen-antibody complex.

The Neisser-Wechsberg Phenomenon (Complement Deviation). It was observed by Neisser and Wechsberg that when varying amounts of immune serum (amboceptor) are added to constant amounts of normal serum (complement) and antigen, there is, as might be expected, no lysis with very small amounts of amboceptor, but as larger amounts are added, lysis takes place. When, however, amboceptor is added in considerable excess, lysis again fails to take place. From these and similar experiments it was concluded that a "deviation of complement" occurs under conditions where the amboceptor is in great excess, i.e., the complement unites with the unbound amboceptor rather than with the amboceptor which has united with the antigen. A special explanation need not be devised for this phenomenon, however, for a similar failure of antigen-antibody union to occur is apparent in other serological reactions in the region of antigen or antibody excess. Such, for example, is the prozone phenomenon (p. 301) in the precipitin and agglutination tests. The same effect may be observed *in vivo*, for successful protection of mice against pneumococcus infection has long been known to be dependent upon optimal amounts of antiserum and culture; otherwise a zonal phenomenon in which there is no protection (the *Schwellenwert* of Neufeld and Haendel) may be encountered.

The Bordet-Gengou Phenomenon or Complement Fixation. As pointed out above, the lytic action of an immune serum is difficult to observe with many bacteria and cannot take place with an antigen such as egg albumin. Whether or not the phenomenon of lysis occurs or is made evident through some observable change in the antigen, it is possible to demonstrate the union of antigen and antibody by the addition of an indicator system. The lysis of red blood cells is used for this purpose and is commonly referred to in this connection as the hemolytic system; sheep cells and sheep hemolysin are generally used.

The antigen and inactivated serum (either of which may be unknown) are mixed together with the proper amount of complement in the form of fresh, unheated guinea pig serum. If the antigen and the inactivated serum unite, the complement will combine with the sensitized antigen and is said to be "fixed." When the hemolytic system is added in the form of heated hemolytic serum and erythrocytes, no complement is available, hemolysis does not occur, and the test is positive. If, however, the antigen and inactivated serum do not react, the complement remains free and combines with the sensitized red cells, hemolysis results and the test is negative. This phenomenon may be illustrated in the following way:



The quantitative aspects of the test are obviously of primary importance,

chapters. Intelligent interference with the course of events is impossible without a clear idea as to what is actually happening.

We may recognize, in theory, at least six categories of hosts among any infected herd. Four of these categories include individuals who are themselves infected, (1) the typical case, (2) the atypical case, (3) the latent infection, and (4) the healthy carrier; the other two categories are not themselves infected, or infective, (5) the uninfected immune and (6) the uninfected susceptible. The division between (2), (3) and (4) is formal rather than actual—these conditions shade into each other by imperceptible degrees. It is doubtful whether the division between (3) and (4) is justifiable even for convenience of description, since many, perhaps most, of the class commonly described as healthy carriers are in reality suffering from a symptomless, and often negligible, infection. It may be noted that the individuals falling in categories (3) and (4) are in general, though in a very varying degree, resistant to further infection from without; so that the only fully susceptible hosts at risk are those in class 6.

Fig. 271 may help the student to visualize the kind of distribution, both of infection and of immunity, that may be met with in infected herds under different epidemic conditions. No distinction has been made in the diagram between latent infections and healthy carriers. A few arrows have been introduced to indicate the direction of effective spread—effective in the sense of producing new cases of disease or in converting susceptibles to immunes. As we shall see, an epidemic of an infective disease is usually accompanied by an epidemic of symptomless immunization.

In this figure, A may be taken as an example of an epidemic phase in an endemic-epidemic prevalence, that is, as representing the state of affairs during an outbreak of an infective disease from which the affected herd is never completely free, epidemics of varying severity recurring at more or less frequent intervals. B may be regarded as a later stage of A, or as a small epidemic wave occurring in a herd in which susceptibles are few, while carriers are frequent. C may be taken as representing the state of affairs during a severe epidemic occurring in a herd with little initial immunity. An extreme example of this catastrophic type of prevalence has occasionally been afforded by the introduction of such a disease as measles into an isolated island community that has either never experienced the infection before, or has been free from it for many years. D may be regarded as representing a stage of relative quiescence between two outbreaks of the type depicted in A. It will be noted that the proportion of susceptibles is higher than in A or B, and with such a distribution as this a fresh outbreak of the A type is likely to occur.

Herd Immunity in Diphtheria.

As an illustration of the types of distribution depicted in A, B and D, no better example could be selected than that of diphtheria. We are here dealing with a disease that is essentially a toxæmia; and, as we have seen, an effective antitoxic immunity will in this case protect the host against clinically detectable infection. In the Schick test we have a method which allows us to divide the members of any herd into susceptibles and immunes. Except in the case of very young children a negative reaction may be taken as an indication that an individual has something over 0.01 A.U. of antitoxin per ml. of circulating blood, and also that he will produce further antitoxin briskly and effectively in response to any entry of toxin

and each reagent must be titrated and added in the proper amount. As usually carried out, the degree of hemolysis is estimated as O, +, ++, +++ or ++++, the last representing complete hemolysis. A considerably more accurate end point is that of 50 per cent hemolysis, measured in a photoelectric colorimeter or spectrophotometer. A quantitative complement fixation which allows a close approximation of amount of antibody has been developed,³⁶ in which an excess of complement is added to the antigen-antibody mixture and the amount remaining after fixation titrated with the usual hemolytic system to a 50 per cent end point.

The complement-fixation test may be used with a known antiserum for the identification of an unknown bacterium or with a known antigen as a means to detect antibodies in an unknown serum. Perhaps the commonest application of the test is in the diagnosis of syphilis, where it is known as the Wassermann test.

*The Nature of Complement.*³⁷ It is likely that the process of lysis is accomplished by complement, the antibody simply sensitizing the antigen to this lytic action. Complement is not increased during immunization, and a higher titer immune serum may be only feebly lytic because there is not sufficient complement present to utilize the excess of amboceptor. It is of some interest that there is little species specificity in complement; that from guinea pig serum, for example, may bring about the lysis of beef corpuscles in the presence of amboceptor in goat serum. Some quantitative differences are apparent, however, for a serum which may be actively complementary in some reactions may be relatively inactive in other combinations.

The property of inactivation by heat has been referred to above. Complement is also inactivated by shaking, but in neither case is the inactivation completely irreversible, for some activity may be regained on standing. In this respect complement behaves as a typical hydrophilic colloid which can be made to aggregate by physical forces but shows a tendency to spontaneous dispersion and restoration to the original state. The activity disappears upon standing—rapidly at room temperature (two or three hours) and more slowly in the ice-box, where it may be preserved for three or four days. Complement is irreversibly destroyed by strong acids or alkalis and reversibly inactivated by ions such as Mg, Ca, Ba, Sr and SO₄ or by hypertonic salt solutions. In this connection it is of some interest that complement which is inactivated by raising the salt concentration to 5 to 10 per cent may be preserved at low temperatures in this form for several weeks, the activity being regained upon dilution with distilled water. These properties and some others, such as ready adsorption on surfaces, suggest a close relationship between complement and the enzymes; it has also been pointed out that there are remarkable resemblances to certain compounds of protein with soaps and lipids. An association of complement with the blood-clotting mechanism is suggested by the observations that many substances have both anticoagulant and anticomplementary activity, and that there is a close correlation between thromboplastic and complement-fixing power of tissue ex-

³⁶ Mayer, Osler, Bier and Heidelberger: *Jour. Immunol.*, 1948, 59, 195.

³⁷ See the review by Pillermer. *Chem. Rev.*, 1943, 33 1.

of events in any particular community that is vastly more informative than we can at the moment supply for most other diseases.

It has of course long been recognized that the diphtheria bacillus is not confined to those who are suffering from the clinical disease in its typical form. It is frequently isolated from cases of mild sore throat associated with an epidemic of typical diphtheria, less frequently from healthy contacts, and still less frequently from non-contacts.

Kober (1899) records the isolation of diphtheria bacilli from 70 per cent. of 139 contacts who were themselves suffering from mild sore throat, and from 8 per cent. of 123 contacts who had apparently normal throats. Closeness and continuity of contact, here as in other diseases, have a considerable influence on the carrier rate. The collected figures recorded in the Medical Research Council's monograph on diphtheria (see Monograph 1923) show a 15 per cent. carrier rate of virulent diphtheria bacilli among 610 contacts in barracks or hospital wards, a 7 per cent. carrier rate among 10,883 home contacts, and a 0.6 per cent. carrier rate among the general non-contact population.

As a measure of the risk to which an ordinary urban population is submitted we may take the carrier rate of virulent diphtheria bacilli among children attending the elementary schools in and about London. During the third decade of the century it fluctuated between 2.5 per cent. and 5 per cent. (see Dudley 1923, Forbes 1927).

The events that follow the passage of virulent diphtheria bacilli from the throat of a case, or of a carrier, to the throat of a non-infected person will depend on the immunological condition of the recipient. If he is susceptible (Schick-positive) he will either respond by developing antitoxin and so becoming more resistant, and eventually Schick-negative, or else he will develop clinical diphtheria. Hence Schick-positive carriers of virulent diphtheria bacilli are very rare. They are not non-existent—they cannot be if we accept the view that the transition from the susceptible to the immune class is usually the result of latent infection—but we may take it that a person who is carrying virulent bacilli in sufficient number to be detectable by an ordinary swabbing is (a) immune (Schick-negative), or (b) undergoing rapid immunization to the Schick-negative level, or (c) incubating the disease.

If, then, we take the case of a relatively isolated community, such as a large boys' school, and trace the spread of infection and the development of immunity during an epidemic of diphtheria, we shall observe the following sequence of events.

At the start we may suppose that we have 50 to 70 per cent. Schick-negative immunes and 30 to 50 per cent. Schick-positive susceptibles. Among these boys there will be a certain number of carriers of virulent diphtheria bacilli, say 3 per cent.; these will be immunes.

When these bacilli spread to uninfected boys they may obtain lodgment either in a susceptible or in an immune; and, depending on the dose of bacilli transferred, the exact degree of immunity of the recipient and many other factors of which we have as yet no knowledge, they may bring about any of the following transformations.

Frequently:

- (a) Schick-positive susceptible → case (mild or severe).
- (b) Schick-positive susceptible → Schick-negative immune
 - (a) without detectable carrying,
 - (b) with detectable carrying.
- (c) Schick-negative immune → Schick-negative immune carrier.

tracts and sera. It seems clear, however, that complement is not identical with prothrombin, and more recent work³⁸ suggests that inactivation of complement of plasma blocks the conversion of prothrombin to thrombin. The nature of the lytic action of complement is as yet, however, purely speculative.

Complement is intimately associated with the serum proteins and there is reason to believe, viz. the destruction of complement by trypsin and the antigenicity of the activity, that this lytic agent is protein in nature. Complement may be split into two parts by the separation of serum protein into albumin and globulin. The globulin fraction will unite with sensitized cells although no lysis occurs, and is called the *mid-piece*, while the albumin fraction which will not unite with the sensitized antigen in the absence of the mid-piece but produces lysis when it unites with the sensitized cell-mid-piece complex is termed the *end-piece*. Other investigations have indicated that complement may be split up into four components by other fractionation methods. These are:

C_1 or mid-piece which is precipitated from guinea pig serum by passing CO_2 through the serum diluted 1:10 with distilled water, or by dialysis against distilled water. This component is destroyed at $56^\circ C$ in 30 minutes and is a euglobulin containing carbohydrate.

C_2 or end-piece which remains in solution after the C_1 fraction is precipitated. It shows the same heat lability and is a part of a mucoglobulin fraction containing 10 per cent carbohydrate.

C_3 is inactivated by yeast, zymin or cobra venom and is not destroyed at $56^\circ C$ in thirty minutes.

C_4 or fourth component is specifically inactivated by treating guinea pig serum with dilute ammonia, hydrazine or viper venom, or by shaking with chloroform or ether. It is also heat-stable but this stability is greatly reduced in the presence of 10 per cent sodium chloride. It appears to be a carbohydrate portion of the mucoglobulin fraction containing C_2 whose carbonyl groups are attacked by ammonia.

Human complement likewise consists of these four components and they are mutually interchangeable with those in guinea pig serum.³⁹ The four components are also present in frog serum and carp serum and are either identical or closely related to those of guinea pig serum.⁴⁰ The titer of complement is, of course, limited by the fraction occurring in smallest amount; this appears to be C_2 in human serum, and C_3 in guinea pig serum. All four components are necessary for lysis of red cells and bactericidal action, but all are not equally fixed by the antigen-antibody complex. In general, relatively large amounts of C_1 and very small amounts of C_3 are fixed, but the relative fixation depends upon what other components are present.

Opsonins. If a mixture of polymorphonuclear leucocytes and bacteria or other particulate matter is incubated for a time, it will be found on microscopic examination that a number of the leucocytes have ingested the foreign particles. Few if any particles will be ingested if the mixture is prepared in physiological salt solution, a considerable number if the fluid is normal serum. In

³⁸ Mann and Hurn Proc. Soc. Exp. Biol. Med., 1948, 67:83.

³⁹ Ecker and Seifter. Proc. Soc. Exp. Biol. Med., 1945, 58:359.

⁴⁰ Cushing Jour. Immunol., 1945, 50 61, 75.

well illustrated by the case rates and carrier rates in one group of some 160 boys during the winter term of 1921 and the winter term of 1922.

		Case Rate.	Carrier Rate.
1921	: : : :	7 per cent.	2 per cent.
1922	: : : :	0.6 " "	6 " "

In 1921 during a severe epidemic, following on a period in which the school had been free from diphtheria for 2 years, the spread of virulent bacilli produced 3.5 cases to one carrier; in the winter of 1922 after this severe exposure, and in spite of the entrance of 43 new boys during the post-epidemic period, the spread of infection produced 10 carriers to one case.

In judging the significance of such figures we must remember that the real carrier:case rate over any considerable interval of time is always higher than that given by comparing the recorded case rate with the recorded carrier rate over that period—a point stressed by Dudley (1932). All clinical cases of diphtheria will be recorded. A case rate of 3 per cent. per annum in a particular community means that 3 persons in every hundred of those exposed to risk develop diphtheria during the year in question. But a carrier rate of 7 per cent. does not mean that 7 per cent. become carriers during the year; it means that at any one swabbing of an adequate sample 7 persons in each hundred are, on the average, found to be harbouring diphtheria bacilli. The total number of persons that become carriers during the period in which the three cases of diphtheria occur will be much higher. Thus, over one yearly period, Dudley records an average carrier rate of 6.6 per cent. with small fluctuations above or below this level; but repeated swabbing (7-8 times) of a large sample of boys showed that at least 40 per cent. were harbouring the diphtheria bacillus at one time or another during this period.

The general significance of the picture presented by such studies as these is clear enough; but we must remember that the two categories—immunes and susceptibles—that are divided from one another by simple Schick testing present a very incomplete picture of the graduations in resistance that actually exist in a herd at risk. Glenny (1925) defines five grades of immunity, which are set out in Table 84. These are, of course, not exhaustive; nor are they sharply demarcated from one another (see also Parish and Wright 1933).

TABLE 84

SHOWING VARYING GRADES OF RESISTANCE TO DIPHTHERIA. (After Glenny.)

	Group				
	1	2	3	4	5
Schick test	~	+	+	+	+
Antitoxin in blood . .	> 1/30 A.U.	< 1/30 A.U.	0	0	0
Earlier stimuli . . .	Many	Many	Many	Few	None
Response to further stimuli	Rapid	Rapid	Rapid	Slow	Very slow
Description	Immune	Immune	Potentially immune	Sub-immune	Fully susceptible

If we could elaborate our study of a population at risk by estimating at regular intervals the exact amount of antitoxin in each person's circulating blood; and if, dealing with a very large population, we could withdraw an adequate sample of persons at frequent intervals and determine the response of each Schick-positive person to small injections of toxoid, we should be able to build up a far more detailed picture of what was really happening. Adopting Glenny's classification, we should be able to detect the transformation from the fully susceptible to the sub-immune, and thence through the Schick-positive immune to the fully developed Schick-negative immune, as well as transference from the Schick-positive to the Schick-negative class.

the case of bacteria, great numbers of the microorganisms will be found packed into the leucocytes when the two are suspended in the specific immune serum. The antibodies present in the immune serum which so remarkably stimulate this engulfment by body cells are designated *bacteriotropins*, a term not in common use, or *opsonins*. The term opsonin was originally used to designate the activity of normal serum, hence the antibodies in the immune animal are sometimes called immune opsonins. The cells which ingest such particulate matter are termed *phagocytes* (devouring cells). This property is not confined to the polymorphonuclear leucocytes or heterophils, although these have been most widely used in *in vitro* experiments because of their availability, but is



Fig. 34. Phagocytosis of pneumococci by culture macrophages. 1, Phagocytosis in presence of normal serum, relatively few bacteria have been ingested. 2 and 3, Phagocytosis in the presence of antipneumococcus serum, enormous numbers of pneumococci have been ingested and appear as agglutinated masses. The lightly staining forms are degenerating. Hematoxylin and eosin-azure II. $\times 1200$ (Zuckerman).

also present in various mononuclear phagocytes. These cells and their role in immunity are discussed in the following chapter.

The Opsonic Index. A quantitative estimate of opsonin present in a given immune serum may be made by comparing the number of bacteria ingested by normal leucocytes in normal serum with the number ingested by normal leucocytes in the immune serum. The appropriate mixtures are prepared and incubated in capillary tubes, smears made and stained, and the bacteria engulfed by an arbitrary number (usually 50 or 100) of leucocytes counted.⁴¹ The average number of bacteria per leucocyte, or *phagocytic index*, is determined for the normal and immune sera, and the ratio of the phagocytic index of the immune serum to that of the normal serum is termed the *opsonic index*.

⁴¹ The details of this technique may be found in Wright and Colebrook: *Technique of the Teat and Capillary Glass Tube* 2nd ed. Constable and Co., Ltd., London, 1921; Fleming: *A System of Bacteriology*, Medical Research Council, London, 1931, 9 212.

of the same general kind as that now available in the case of diphtheria; but we have not yet any records comparable in detail and duration with those afforded by Dudley's work at Greenwich. So far as our fragmentary records go they look like pieces of a very similar picture, with one essential difference. The production of the erythrogenic toxin forms a relatively small part of the pathogenic potentialities of hæmolytic streptococci; and an antitoxic immunity that will suffice to prevent the occurrence of the clinical syndrome that we label scarlet fever will not suffice to prevent the spread of tonsillitis, or of other clinically obvious streptococcal infections (see Okell 1932). For effective immunity against all the clinical manifestations of infection with hæmolytic streptococci we need an antibacterial as well as an antitoxic immunity; and this antibacterial immunity will almost certainly be type-specific.

Turning to diseases in which antitoxic immunity, in the strict sense in which we are using that term, plays no apparent part, we are not in a position to give any adequate description of the course of events in a naturally infected herd. This is because we have no such simple tests as the Schick and Dick reactions by which to separate our immunes from our susceptibles. There are good reasons for believing that by an adequate survey, including a careful study of the distribution of the infecting organism and tests for the presence of specific antibodies in the blood of the hosts at risk, we could add greatly to our knowledge of what is happening during an endemic or epidemic prevalence. But we have not got that knowledge yet.

Some things we do know. We know that in certain diseases, such as cerebrospinal meningitis, the ratio of carriers to cases is very high (see for instance Glover 1918), so that it seems probable that the immunes greatly outnumber the susceptibles. The same general relationship—a widespread carrier epidemic associated with relatively few clinical cases—seems to hold in certain virus diseases, such as poliomyelitis and encephalitis lethargica.

In other diseases, such as typhoid fever, the ratio of carriers to cases is not so high (see Chapter 69). But we know that atypical cases of typhoid fever occur during an epidemic, and that many carriers of typhoid bacilli give no history of ever having suffered from a typhoid-like disease. It is a fairly safe assumption that an endemic or epidemic prevalence of typhoid fever is associated with the occurrence of sub-clinical immunizing infections. We know quite certainly that such a prevalence leaves behind it infected healthy carriers who may be a potent source of further spread.

THE EXPERIMENTAL STUDY OF HERD INFECTION AND IMMUNITY

It is clearly possible, by selecting a convenient host species and a parasite that spreads naturally among them, to submit problems of the kind we have considered above to direct experimental study—initiating an epidemic of a particular disease among our test population, and studying the reactions of new entrants, of old members of the herd, or of migrants from one herd to another, by any available means (see Greenwood *et al.* 1936). We can, under such conditions, observe the effects of any intentional interference we choose, splitting a herd into smaller units, reaggregating these units after any selected interval, varying the rate of

The opsonins may be estimated also by the dilution method; specimens are prepared in the usual way, except that the normal and patient's sera are diluted with saline or Ringer's solution. One mixture is made with salt solution or Ringer's solution to determine the degree of spontaneous phagocytosis. The dilution of serum which gives the same amount of phagocytosis as a mixture without serum is taken as the end point.

The Factors Influencing Phagocytosis. The process of phagocytosis is markedly influenced by environmental factors and the nature of the bacterium and leucocytes used, as well as by the amount of opsonin present. Departures from a neutral or very slightly acid reaction or from isotonicity, and the presence of certain ions, notably the citrate radicle, depress the degree of phagocytosis. The last is of practical significance in that it contraindicates the use of citrated blood. The presence of calcium, on the other hand, may restore phagocytic power to leucocytes which have been allowed to stand in isotonic salt solution for a number of hours, a point of some interest in connection with the apparent intimate relation between this ion and cell division and the ability of ameboid cells to form new surfaces.

The nature of the bacterium to be ingested is of considerable importance; in general, virulent forms are relatively resistant to phagocytosis. It is not unlikely that this resistance is associated with the presence of a capsule, in the case of the pneumococcus, for example, not only are the smooth, encapsulated forms highly resistant to phagocytosis, but the presence of capsular polysaccharide markedly inhibits phagocytosis by an immune serum, presumably through combination with the antibody. Bacteriophage (p. 916) renders bacteria considerably more sensitive to phagocytosis.

The phagocytic power or activity of the leucocytes is subject to considerable variation independent of variation in the opsonic content of the blood. This inherent phagocytic power of the leucocytes varies, with respect to certain bacteria at least, even in persons apparently in perfect health. In the child at birth the leucocytes are somewhat less active phagocytically than in the adult; they grow less active for a few months, and then more active, the adult standard for streptococci, pneumococci and staphylococci being reached about the third year. In pneumonia, scarlet fever and other conditions in which there is acute leucocytosis when the outlook is favorable, the phagocytic power of leucocytes has been found to be greater than normal for the specific bacteria. The increase in activity in such cases may be due to the predominance of young leucocytes.

The Process of Phagocytosis. The mechanism of ingestion is essentially one of the interplay of interfacial forces. A formulation of the free surface energy relationships at the points of contact between particle and cell and their respective liquid interfaces has been worked out by Fenn⁴² and subjected to experimental tests by Mudd⁴³ and his associates. Low interfacial tension of the bacteria against the leucocytes—and high interfacial tension against the medium—favors ingestion. The immune opsonins, and to some extent the

⁴² Fenn: *The Newer Knowledge of Bacteriology and Immunology*. Jordan and Falk, University of Chicago Press, Chicago, 1928. p. 861.

⁴³ The process of phagocytosis is discussed at length by Mudd, McCutcheon and Lucke. *Physiol. Rev.*, 1934, 14 210.

Similarly (Greenwood *et al.* 1936), the results obtained in experiments with the virus disease, ectromelia, indicated that some 80 per cent. of the mice entering the herd were infected with the virus within three weeks of entry (see also Fenner 1949b).

The way in which the bacterial parasites are distributed among the hosts at the time of infection is of course of great importance in determining the results in immunization as well as, or in place of, the production of overt disease? That survivors from an epidemic are, on the average, more resistant than new-comers to an infected herd is certain; for they live longer—usually much longer—when exposed to a subsequent wave of mortality (Topley 1921, Amoss 1922).

This increase in resistance with increasing herd experience under epidemic conditions can be studied in greater detail by constructing life tables for the mice submitted to risk of infection during a long-continued prevalence of such a disease as mouse typhoid. It is convenient in calculating the expectation of life to limit it to 60 days, regarding any mouse living for longer periods as dying on the 60th day, since the use of the unlimited expectation of life gives undue weight to the relatively few mice that live for much longer periods, and also because we have a normal standard of comparison for the shorter period, but none for the latter.

Employing this value ($_{60}E_x$) we find in a particular experiment, in which 3 normal mice were added to an infected herd each day from March 6th, 1921, to June 30th, 1923, that the limited expectation of life drops slightly (from 22.49 to 21 days) during the first week of herd life and then rises, at first slowly, but more steeply after the 15th day, until by the 33rd day it reaches the figure of 34.75 days. It fluctuates round this figure up to the 100th day of herd residence; after which the numbers of survivors are too small to give a reliable average. In general then, we may say that a mouse that has survived about 30 days' exposure to risk will live more than half as long again as a new-comer to the cage (Greenwood and Topley 1925, Greenwood *et al.* 1936).

We can ask the same question in another way, using a direct experimental comparison instead of a life table. In a particular experiment (Greenwood, Newbold, Topley and Wilson 1926) in which mouse pasteurellosis was the infection selected for study, two parallel epidemics (A and B) were initiated on November 11th, 1924, and were maintained by the addition of normal mice until December 1925. At regular intervals groups of mice that had survived for 10, 20, 30, 40, 50 days or more in Herd A were transferred to Herd B, and with them were added a numerically equal group of normal mice that had had no previous experience of pasteurellosis. Averaging the results, the figures shown in Table 85 were obtained:

TABLE 85

Length of Life in Herd A.	Expectation of Life in Herd B ($_{60}E_x$).
Nil (normal entrants to B)	22.37 \pm 0.36 days
10 days	21.34 \pm 1.10 "
20 "	25.50 \pm 1.13 "
30 "	32.55 \pm 1.28 "
40-45 days	33.08 \pm 1.52 "
50-60 "	37.39 \pm 1.69 "

Here, again, survival through a testing period of 30 days or more added some 50 per cent to a mouse's expectation of life under severe epidemic conditions.

But clearly we cannot, in the absence of further evidence, assume from such

"normal" opsonins, apparently form a surface deposit on the bacterial cells which promotes phagocytosis by altering the interfacial tension in this manner. In addition to surface tension, other factors such as the viscosity of the phagocytic cell substance enter into this phenomenon. Physical tensions are not the sole controlling factors, however, for increased oxygen consumption accompanies the process of ingestion, the rise beginning at once, reaching a maximum value twice that at the start in about fifteen minutes and persisting for 90 to 150 minutes.

It is of some interest that bacterial cells may be artificially "opsonized," i.e., made more readily phagocytatable, by treatment with iron ammonium alum, chrome alum, protamine sulfate or gallotannic acid. The effect is reversed by treatment with oxalate but reversion is very difficult when the cells are sensitized with immune opsonin. The significance of such observations in immune opsonization and phagocytosis is not clear.

The work of Wood and his co-workers⁴⁴ has shown that phagocytosis may occur quite as readily in the absence of antibody as in its presence, provided that it occurs on a suitable surface; most body tissues provide such surfaces, viz., the phagocytosis of pneumococci on the alveolar surfaces of the lungs. This phenomenon of "surface phagocytosis" has not been previously described.

The Fate of Ingested Bacteria. Following phagocytosis many, but not all, species of bacteria may be observed to undergo a process of dissolution, with swelling, granulation and fragmentation appearing as successive stages in their destruction. Although it was early supposed that intracellular digestion was no more than intracellular lysis through the agency of the immune lysin and complement which would have occurred whether or not phagocytosis took place, it now seems probable that the two processes are essentially different.

Bacteria are no exception to the rule that living organisms are not subject to attack by digestive enzymes, and the question arises as to whether death is a necessary preliminary to ingestion or whether it may occur within the phagocyte. It is probable that the viability of the microorganism is not an important factor in phagocytosis; in most cases the cell may be engulfed whether it is living or dead. In the case of those bacteria which are destroyed within the leucocyte, killing is necessary before digestion can take place. Killing of a viable bactericidal substance not equally viable within bacteria are

protected against the action of immune serum, i.e., bactericidal or lytic antibodies, and this is possibly of some significance in the dissemination of bacteria within the host in the event that they remain viable and are later freed from the leucocyte. Leucocytes have been shown to contain a variety of digestive enzymes, including proteases, lipases and various carbohydrate-splitting ferments which are presumably responsible for the actual dissolution of the bacterial cell.

The Nature of the Opsonic Activity. The opsonins are true antibodies in that they are increased by immunization and exhibit the specificity characteristic of the immune antibodies. The activity is, like that of the lytic sera, made

⁴⁴ Wood, Smith and Watson: Science, 1946, 103 28.

that entered herd B on the same days as these particular groups of migrants from herd A was 20.9 ± 0.49 days; for those migrants that had spent 50 days in herd A it was 34.6 ± 4.10 days.

It may be noted also that such evidence as is available (Topley, Wilson and Lewis 1925, Greenwood and Topley 1925) indicates that the increased resistance gained by survival in an infected herd, or after experimental infection by feeding or inoculation, is specific in the ordinary bacteriological sense. A mouse that has survived infection with *Salm. typhi-murium* appears to be no more resistant than normal mice to infection with *Pasteurella muriseptica*. This, of course, accords well with epidemiological experience in general, but not with the view that the determining factor in increasing the average resistance of a herd at risk is the selection by the sieve of death of those individuals possessing an innate non-specific resistance. If differences in innate resistance are of major importance it would appear that they must either be specific or must be differences of immunizability rather than of immunity.

Whether or not active immunization is the main factor that determines the increased average resistance of surviving mice, the grade of antibacterial immunity produced can excite little enthusiasm.

As stated above, the expectation of life limited to 60 days was selected in these and subsequent experiments, in part because there is an available standard of reference for this period (Greenwood, Topley and Wilson 1931b). On October 4th, 1929, 20 normal mice were assembled in a cage of the same type as that used in all epidemic experiments. From then onwards until January 17th, 1930, daily additions of normal mice were made, and the herd was then observed until May 27th, 1930. The 329 mice of this experiment were living under exactly the same conditions as the infected herd, except that no infection was present at any time during the period of observation. Of these 329 mice 56 died. The limited expectation of life, as was to be expected, remained practically constant at all cage ages from day 0 to day 170—the last day for which figures were available. The lowest $_{50}E_x$ figure was 56.69 days, the highest 59.27 days. The figure rose slowly from just under 57 days on the day of entry to over 58 days on the 40th day of cage life—presumably as the animosities of first acquaintance were replaced by the toleration of later herd life and fighting grew less frequent. Thereafter it hardly varied.

Taking a figure of 58 as our normal $_{50}E_x$, we note that the best that natural immunization against mouse typhoid or mouse pasteurellosis can do—so long as surviving mice are exposed to a continuous risk of heavy infection—is to raise the expectation of life from a little under a half to a little over two-thirds of the normal figure. This is a very different picture from that presented by our study of diphtheria in man—even allowing for the fact that in the latter case we are dealing with a disease of relatively low fatality. Either mice differ in some fundamental way from men; or the conditions in mouse cages differ fundamentally from those in schools and institutions—an obvious possibility; or natural immunization against enteric infection or against pasteurellosis is less effective than natural immunization against diphtheria or scarlet fever. Perhaps, though by no means certainly, this might be expanded to the conclusion that natural antitoxic immunization is much more effective than natural antibacterial immunization.

There is no known disease of mice that presents any close analogy with such toxæmic human infections as diphtheria and scarlet fever; but the description by Marchal (1930) of a natural virus disease of mice—ectromelia—that gives rise to severe and fatal epidemics under experimental conditions has afforded an opportunity for the study of natural immunization against a virus infection. The results

up of two components, one thermostable and the other thermolabile. The thermolabile component is present in normal serum and the reduced opsonic powers of an inactivated immune serum may be restored by the addition of a small amount of normal serum. In this and other respects the thermolabile component of opsonin strikingly resembles complement and in the past has been assumed by many to be identical with this component of the lytic system. In general, however, it has not been possible to show any constant relation between the components of complement and opsonization. For example, the fourth component of complement has been reported⁴⁵ to be necessary to the lytic reaction but not required for opsonization, but Ecker⁴⁶ has reported that the thermolabile opsonin of the human serum is identical with C'_1 , C'_2 and C'_4 in combination but not separately or in any combination of two. It ap-

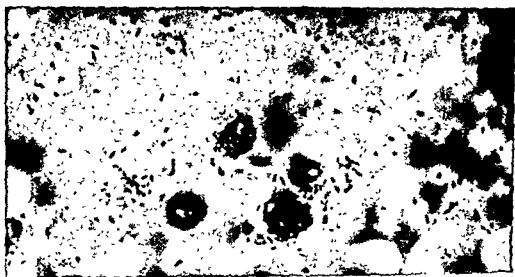


Fig 35. The phagocytosis of typhoid bacilli by leucocytes in whole blood. Note the enormous numbers ingested by the white cells and the bacilli lying free. Hastings' stain; $\times 1200$.

pears, then, that complement and the thermolabile element of opsonin, while resembling each other closely in many respects, may not be regarded as identical.⁴⁷

There is some question as to whether the thermolabile factor is essential to phagocytosis, for experimental evidence has been presented⁴⁸ which indicates that while the thermolabile factor contained in normal serum markedly accelerates the rate of phagocytosis, approximately the same number of bacteria are ingested at the end of eight hours by phagocytes in the presence of heated serum alone.

Agglutinins. If the blood or serum of an animal previously immunized against a bacterium be mixed with a suspension of the microorganisms, the latter become immobilized and in a short time aggregate to form large clumps of cells. In the test tube these clumps settle out, and the turbid bacterial sus-

⁴⁵ Gordon, Whitehead and Wornall: *Jour. Path. and Bact.*, 1929, 32:57.

⁴⁶ Ecker and Lopez-Castro: *Jour. Immunol.*, 1947, 55:169.

⁴⁷ See also Ecker, Pillemer and Kuehn: *Jour. Immunol.*, 1942, 43:245.

⁴⁸ Ward and Enders: *Jour. Exp. Med.*, 1933, 57:527.

mice showed a partial but ineffective immunity in that they lived on the average far longer than the controls but succumbed to the disease during the period of observation; 24 per cent. showed a more effective resistance by living in apparent health throughout the period of observation, but post-mortem examination showed that they were suffering from a latent infection; 18 per cent. appeared to have been rendered completely immune to this dose of bacteria, in that they remained in apparent health for 28 days and showed no evidence of infection when killed and examined by spleen culture. These results may be taken as representative of a considerable number of similar experiments.

TABLE 87

	No Inoculated.	No Dead in 28 Days	Average Time to Death of Mice that Died.	No Survivors with Positive Spleen Cultures
Normal	50	50	5.0 days	—
Vaccinated . . .	50	29	18.3 "	12

We should not perhaps expect an immunity of this order to be very effective as a prophylactic measure in herds submitted to a continuous risk of infection, and actual trial confirms our lack of faith.

An epidemic of mouse typhoid was started on January 1st, 1928, and was continued until September 17th, 1929, by the addition of 3 normal mice a day (Greenwood, Topley and Wilson 1931a). Every 7th day 10 additional normal mice, and several groups, each of 10 mice, that had been immunized with different vaccines were added to the cage. The results, as they concern the relative efficacy of different antigenic components, have already been noted on p. 1201. We are concerned here only with the results obtained with the most effective vaccines employed.

The limited expectation of life ($_{60}E_x$) of the normal mice on entry was 26.26 ± 0.641 days; for the four immunized groups that had received vaccines containing the smooth surface antigen the $_{60}E_x$ figures were 32.10 ± 0.432 , 34.97 ± 0.845 , 35.06 ± 0.845 and 33.71 ± 0.853 days respectively. Thus by active immunization with a killed vaccine an increase in average resistance was obtained of the same order as that attained naturally by normal mice after living for some 30-50 days in an infected herd under the joint influence of active immunization and mortality selection.

After 60 days' residence in the cage the $_{60}E_x$ figures for the vaccinated and unvaccinated groups have, as we should expect, largely levelled up, though the vaccinated mice still show a slight advantage. The figure for the unvaccinated group has risen to 36.47 days, those for the vaccinated groups to 40.17, 37.39, 36.90 and 47.54 days respectively.

Very similar results were obtained in another experiment in which a comparison was made between the effect of administering a killed *Salm. typhi-murium* vaccine by the mouth, or by intraperitoneal inoculation (Greenwood, Topley and Wilson 1931c). A larger dose was used for *per os* than for intraperitoneal immunization ($5,000 \times 10^6$ as against $1,000 \times 10^6$) with a week's interval after the second dose of

vaccine. The experiment lasted from December 14th, 1929, to June 26th, 1930. The death rate in this epidemic was rather lower than in those referred to above, and the expectation of life of the normal mice on entry was therefore somewhat longer. The $_{60}E_x$ figures on the day of entry were as follows: normal mice 31.18 ± 1.04 days, mice vaccinated *per os* 35.30 ± 1.22 days, mice vaccinated intraperitoneally 38.19 ± 1.17 days.

The immunity to ectromelia induced by vaccinating mice with a formalized

pension is cleared with the formation of a precipitate-like mass of clumped cells in the bottom of the tube. This phenomenon is termed *agglutination* and the bacterial cells are said to be *agglutinated*. The bacteria are not killed by agglutination and will, in fact, grow in immune serum although with altered morphology and the formation of long chains of bacillary forms—the so-called “thread reaction” of Pfaundler. Living bacteria need not be used for the agglutination reaction, for dead bacteria are agglutinated as readily as the viable forms.

Although many species of bacteria may be clumped by “normal” sera in low (1:5 to 1:10) dilutions, the capacity of a serum to agglutinate bacteria is greatly enhanced by immunization; high-titered antisera may be prepared which will bring about agglutination in dilutions of 1:20,000 to 1:50,000. The agglutination reaction is, then, an antigen-antibody reaction and the antibody is designated an *agglutinin*. The antigen is sometimes termed an *agglutigen*. The agglutinin does not require the cooperation of complement or other heat-labile substances and inactivated sera will agglutinate to titer.

Not only bacteria but a variety of free cells, including erythrocytes and others, are agglutinated by normal and immune sera. The incompatibility of human blood groups is a consequence of the presence of hemagglutinins. Hemagglutinins are also formed by some bacteria and may possibly be instrumental in the formation of the thrombi observed in the blood vessels after death from certain of the infectious diseases.

the cooperation of a heat-labile component. The reaction may be observed microscopically by mixing a suspension of bacteria and diluted antiserum on a slide but is most commonly carried out by mixing the two in 0.5 to 1.0 ml. amounts in small test tubes and observing the formation of a precipitate. In the latter instance, varying dilutions of serum (frequently prepared in geometrical progression by mixing with an equal amount of physiological salt solution, i.e., 1:10, 1:20, 1:40, 1:80, etc.) are added to the bacterial suspension and incubated at 37° overnight or at 55° C. for two hours. The highest dilution showing observable flocculation is taken as the titer of the serum; a serum showing agglutination in a dilution of 1:10,000 but not in 1:20,000 is said to have an agglutinin titer of 1:10,000. Such titers are variable to some degree depending upon the density of the bacterial suspension and other factors, light suspensions, for example, showing only a faint turbidity to the eye will give higher agglutinin titers than heavy suspensions.

Cross Reactions. Although the agglutination reaction is highly specific, certain cross reactions between closely related bacterial species are frequently observed. Such reactions are attributable not to a lack of immunological specificity but to the immunological heterogeneity of the bacterial cell. As indicated above, the cell is made up of a variety of antigenic components, an “antigenic mosaic.” Clearly, then, if the same component is present in each of two species of bacteria, an antiserum prepared against the one will agglutinate the other but generally to a reduced titer, i.e., only in the lower serum dilutions. This sharing of antigenic components and the resulting cross reactions

kind to that of intraperitoneal immunization, though the resistance induced is slightly inferior.

In the case of the virus disease, ectromelia, the natural immunizing response is far more effective. The immunity attained is not complete—old survivors sometimes die of typical ectromelia, even though they have passed through a charac-

TABLE 88

SHOWING LIMITED EXPECTATION OF LIFE ($_{40}E_x$), IN DAYS, FOR VARIOUS GROUPS OF MICE SUBMITTED TO EPIDEMIC INFECTION.

Infection.	Normal.		Vaccinated.			
	N.E.	S.	I.P.		P.O.	
			N.E.	S.	N.E.	S.
None	58 00	58 00	—	—	—	—
Mouse Typhoid (a) . . .	22 49	31 75	—	—	—	—
Mouse Typhoid (b) . . .	26 26	36 47	32 10 35 06	40 17 36 90	—	—
Mouse Typhoid (c) . . .	31 18	42 40	38 19	49 52	35 30	44 87
Pasteurellosis	22 37	37 39	—	—	—	—
Ectromelia (a)	30 71	54 69	—	—	—	—
Ectromelia (b)	20 8	52 8	49 1	51 6	—	—

I.P. = Intraperitoneal.
N.E. = New Entrants.

P.O. = *Per os*.
S. = Survivors after 30-100 days.

teristic attack many months earlier—but it is of a high order. In conformity with this, vaccination with a formolized virus greatly increases the expectation of life of the mice exposed to risk.

It is clearly of interest to determine the effect of immunizing all entrants to an infected herd. Will this procedure result in the elimination of the disease, at least in its overt form? In the particular case of mouse typhoid, it will not.

In an experiment designed to test this point (Greenwood, Topley and Wilson 1931c) 3 normal mice were added daily to an infected herd from March 15th, 1929, to May 12th, 1929. The limited expectation of life on entry of these mice was 27.00 ± 1.019 days. From May 13th, 1929, to October 20th, 1929, 3 immunized mice were added daily instead of the 3 normal mice, and from October 21st, 1929, to June 29th, 1930, 1 immunized mouse

the three-a-day period was 34.92 ± 0.684 days, for those added during the period it was 36.38 ± 0.938 days—results very similar to those recorded above. But mouse typhoid was spreading and killing as actively in June 1930 as it was in March 1929. The mice at risk lived a little longer, but that was all.

It would, of course, be quite wrong to conclude—even if we may justifiably argue from mice to men—that active immunization of such a kind is useless. As has been noted above, it is capable of completely protecting a proportion of mice

are particularly common among the paratyphoid *Salmonella* and dysentery bacilli. The agglutinins responsible for such cross reactions are sometimes referred to as "group agglutinins" and the phenomenon as "group agglutination."

Agglutinin Absorption. The direct demonstration of the antigenic heterogeneity of a bacterium and the consequent multiple antibody content of its homologous antiserum are made possible by agglutinin absorption. If a heavy suspension of bacteria is prepared in diluted (commonly 1:50) antiserum, incubated for two to three hours, and centrifuged, the supernatant diluted serum will be found to have lost its ability to agglutinate the bacterium with which it was absorbed; the agglutinins for that microorganism have been taken up by the bacterial cells, leaving other agglutinins intact. In practice it is necessary to absorb two or three times and, since the phenomenon is an adsorption, it is not always possible to remove completely the agglutinins in question.

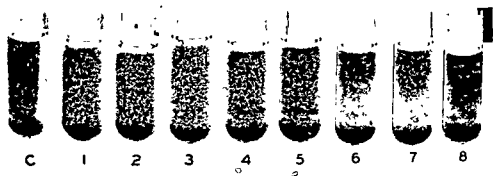


Fig. 36. The macroscopic agglutination test—*Salmonella*. The control tube, C, contains bacterial suspension in the numbered tubes are 1 100, 1:200, 1 500, 1:10,000, 1:20,000. Note agglutination in 1 10,000 but not in 1:20,000.

This preferential absorption makes possible the breakdown of the antigenic mosaic of the bacterial cell into component parts. For example, an antiserum prepared against a bacterium containing antigens A, B and C will contain antibodies *a*, *b* and *c*. If such an antiserum is absorbed by a second bacterium which contains antigens B and C, antibodies *b* and *c* will be removed leaving *a* intact, and the antiserum will still agglutinate its homologous bacterium. Suppose an antiserum is prepared against the second bacterium which will contain antibodies *b* and *c*. If this serum is absorbed by the bacterium containing antigens A, B and C, it will no longer agglutinate its homologous antigen, for all the antibodies will have been removed. Clearly, then, the complete absorption of a known serum by an unknown organism does not indicate that the unknown organism is necessarily immunologically identical with that with which the known serum was prepared; to prove such immunological identity a "mirror absorption" must be carried out, *i.e.*, each antiserum absorbed with each organism.

The agglutination test and agglutinin absorption, although used to some extent in the diagnosis of infectious disease (as in the Widal test in which typhoid bacilli are mixed with patient's serum), have been found particularly valuable in the study of the relation of bacterial species to one another and in

of appeal; but neither from this source nor from any other should we accept hasty judgments.

The experimental data at present available may be summarized as follows:

Webster and his colleagues (Webster 1923*a, b, c, d*, 1924*a, b, c, d*, 1925, 1926, 1927, 1928, 1930*a, b, c*, Webster and Burn 1926, 1927*a, b*) have shown quite clearly that different strains of the same bacterium, isolated at different periods of the same epidemic prevalence, may possess an approximately equal virulence as judged by direct inoculation tests, including inoculation into the stomach through an oesophageal tube. This, indeed, was their constant finding with the strains they examined. They conclude that changes in bacterial virulence play no part in the fluctuations in mortality that may be observed at different stages of a long-continued epidemic prevalence. These they would regard as due to changes in the average host resistance, and particularly to innate differences among the hosts at risk, or to seasonal or dietetic factors, that affect resistance in some non-specific way. They have, however, recorded differences in virulence among strains of the same bacterial species isolated from different epidemic prevalences of the same infective disease (Webster 1930*d*, Hughes 1930, Hughes and Pritchett 1930, Pritchett, Beaudetto and Hughes 1930*a, b*), and they would regard these differences in virulence as determining certain observed differences in behaviour between one epidemic and another—for instance, differences in the proportion of carrier infections to fatal cases of the disease. More recently Webster and Clow (1933) recorded an interesting series of observations on the variations in the virulence of pneumococci for mice. They found that a strain of high virulence, as judged by intraperitoneal injection, might be of low virulence as judged by injection into the nasal cavity; and that a strain of high initial intranasal and intraperitoneal virulence might, as the result of passage from nose to nose, lose all, or almost all, its intranasal virulence, without affecting its intraperitoneal virulence. These findings did not, however, lead them to modify their earlier conclusions. Webster's main contention is that the course of any particular epidemic is determined essentially by the level of genetic resistance of the individuals composing the herd (Webster 1946).

We have so far considered variations in virulence; but virulence may not be the only factor concerned. If we assume the possible existence of another factor, labelling it infectivity and defining it as the capacity to spread from one host to another under any specified conditions of exposure to risk of infection, we may inquire whether infectivity is so highly correlated with virulence that we may regard them as synonymous terms.

The evidence available (Topley, Greenwood, Wilson and Newbold 1928) suggests, as would be expected, that those strains of *Salmonella typhi-murium* that are capable of spreading rapidly among a herd of mice, giving rise to severe epidemics of mouse typhoid with a high mortality rate, are always of relatively high virulence as judged by direct intraperitoneal inoculation, and that strains of low virulence are incapable of giving rise to

infectious, or even to an occasional death, among the normal mice exposed to contact

of this organism, isolated originally from an infected mouse, than an unusual combination of characters (Topley, Greenwood and Wilson 1931). It was of moderately high virulence, killing in repeated tests some 65-75 per cent. of mice inoculated with 100-1,000 bacilli, but it possessed little power of producing severe epidemics by contact infection. In six closed epidemics, in each of which 100 normal mice were exposed to infection from 25 infected companions, the average limited survival time of the exposed mice ($_{50}E_2$) was 51.27 days—not far short of the normal limit. The proportion of the

some instances, as in the case of the *Salmonella* (Chapter 19), complex antigenic formulae have been worked out. The determination of the components of the antigenic mosaic of a bacterial species is termed *antigenic analysis*.

The Prozone Phenomenon. It is not infrequently observed that immune sera showing high agglutinin titers fail to agglutinate the homologous bacteria in low dilutions, i.e., 1:100 or less. This portion of the dilution range is designated as the *prozone* or *proagglutinoid zone*. Prozones, although occasionally observed with fresh sera, are more common and extend over a wider range with old or heated sera. The prozone observed with fresh serum is commonly assumed to be associated with the presence of complement and some other factor, while that shown by heated serum is attributed to the combination of serum albumin with immune globulin or with the antigen-antibody complex in such a way as to prevent aggregation.⁴⁹

"H" and "O" Agglutination. A number of bacterial species, particularly *Salmonella*, *Proteus* and certain others, may agglutinate in one of two ways. Macroscopically the "H" agglutination gives rise to a loose, flocculent precipitate, and upon microscopic examination it may be observed that the bacterial clumps are loose, the flagella of the microorganisms being entangled with one another. The "O" agglutination, on the other hand, produces a finely granular precipitate in which the individual bacterial cells are closely packed together. These types of agglutination have been shown in micromotion pictures by Pijper.⁵⁰ They are a consequence of the presence or absence of the flagellar, heat-labile "H" antigen. Immunization with the whole bacterial cell containing both flagellar and somatic antigenic components gives rise to anti-sera containing both types of antibody, the "H" generally in high titer while the "O" antibody is commonly active in dilutions of less than 1:1000.

Spontaneous Agglutination. Some strains of bacteria do not form stable suspensions and are said to be spontaneously agglutinable. This behavior is particularly characteristic of rough variants and, although not an immunological phenomenon, is frequently of practical importance in agglutination studies.

Cold Agglutinins. Autohemagglutinins which clump red cells at 0° C. but not at 37° C. have been described as occurring in primary atypical pneumonia (p. 870) and a variety of other conditions including trypanosomiasis, mumps, hemolytic anemia. The significance of these agglutinins and their relation to the usual immune agglutinins is not clear⁵¹, possibly the phenomenon is related to the antibody-like activity of so-called acute phase serum (p. 306).

*The Mechanism of Agglutination.*⁵² The clumping of bacteria under the influence of immune serum may be taken as evidence *per se* that a force attracting the cells to one another is operative at least at times. Similarly, the fact that bacteria are not in a constant state of agglutination is indicative of a force which tends to hold the cells apart from one another. The attractive or cohesive force is probably that of surface tension, i.e., the interfacial tension at the cell surface, and the repulsion that of like electrical charges, for, as pointed out previously, bacteria are negatively charged at pH's compatible with viability.

⁴⁹ See the discussion by Hayes: *Erit. Jour. Exp. Path.*, 1947, 2: 98.

⁵⁰ Pijper: *Jour. Bact.*, 1941, 42: 395.

⁵¹ For instance, see Finland, Petersen and Barnes: *Jour. Clin. Invest.*, 1945, 24: 474.

⁵² For the literature to 1919 see Buchanan: *Jour. Bact.*, 1919, 4: 73.

Observations on filtrable viruses parallel in most respects those on bacteria. The main difference appears to consist in the greater lability of the viruses and in the wider range of variations in virulence of a given virus observed under natural conditions.

Fenner (1949b), working with the ectromelia virus, was able to confirm the distinction, observed by Topley and Greenwood in *Salmonella typhi-murium*, between virulence and infectivity. Two strains, for example, had almost the same degree of virulence for mice as judged by direct pad inoculation, yet varied greatly in their power to infect mice under natural conditions. That the difference in infectivity was not due to increased contamination of the environment by one of the strains was shown by exposing mice to the risk of infection with the same number of virus particles. The difference, however, between the two strains was considerably diminished when the infective dose was increased.

Most viruses so far studied can be adapted to different hosts from those in which they are normally found. Sometimes only a few passages through the chick embryo, the mouse, or some other animal are necessary to modify their original characters; sometimes 50, 100 or more passages are required. With this degree of plasticity, it is not surprising that several variants of the same virus are often met with under natural conditions. Good examples are those of foot-and-mouth disease and poliomyelitis; but the most striking example of all, about which we know most, is the influenza virus. The variations of this virus are discussed at some length in Chapter 74 and there is no need to describe them again here. Suffice it to say, however, that from nearly every epidemic of recent years a strain has been isolated differing to a greater or less extent from strains met with previously; and sometimes in the same epidemic strains of different virulence have been isolated, judged both by their behaviour in the human population and in experimental animals (see Wilson Smith 1951, Isaacs, Gledhill and Andrewes 1952). Even in influenza, however, no conclusive evidence has yet been brought to show that the rise and fall of a given epidemic is determined by variations in the virulence or infectivity of the strain responsible.

In summary; variations in virulence in a bacterial parasite during an epidemic prevalence have often been sought for with negative results. There is, however, experimental evidence that different strains of the same bacterial parasite, possessing different degrees of virulence or of infectivity, produce very different results when they are allowed to spread by natural infection among a susceptible herd. There is some evidence that a bacterial parasite may vary in infectivity during an experimental epidemic, though there is as yet no satisfactory proof that the resulting variant influences the progress of the epidemic. Taking the experimental evidence as a whole, it would seem to accord quite well with the view, expressed by many epidemiologists, that variations in the characters of the parasite are of major importance in the spread of human infections, and that an outbreak of disease may be initiated by the evolution, or importation, of an "epidemic strain" of the causative organism. Nevertheless, it seems probable that, with the exception perhaps of certain viruses, the evolution within any parasitic species of a strain of high epidemicity or virulence is an occasional event, rather than part of a normal or periodic process determining the fluctuations in mortality and morbidity that may occur during a long-continued epidemic.

The Role of other Factors in Herd Infection and Herd Immunity.

All those factors that affect the resistance of the individual will affect the average resistance of the herd of which he is a member. Those factors that have been adequately studied have been considered more or less briefly in earlier chapters.

There are, as we have said, special factors, such as the spatial distribution of

On the basis of such reasoning it would appear that the balance between these opposing forces determines whether the microorganisms will form a stable suspension or whether they will clump together and settle out.

It was early observed that the presence of an electrolyte is essential to agglutination; if both immune serum and bacterial suspension are dialyzed free of salt before mixing, the cells are not agglutinated, but if a trace of salt is added to the mixture, agglutination takes place at once. This behavior, it will be seen, corresponds to that of a mixture of two colloids of opposite charge,

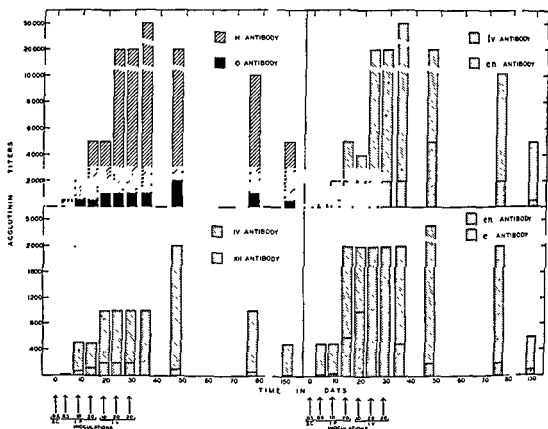


Fig 37. The immune response in the rabbit to the antigenic complex of *Salmonella brandenburg*. Upper left, H and O agglutinin titers, showing the usual marked discrepancy between the two. Lower left, the differential response to the two components of the O antigen. Upper and lower right, the response to the components of the H antigen. Note the independent behavior of the antibodies as indicated by variation in peak titers. All titers determined by direct agglutination of heterologous antigens without agglutinin absorption.

such as gum mastic and gelatin, when the one is added in too small an amount to precipitate in the absence of salt. That the repelling effect of electrical charge is an important factor is also indicated by the agglutination of bacteria in the absence of antibody when the pH is lowered to the isoelectric point of the cells, a phenomenon termed *acid agglutination*. It was formerly thought that bacterial species might be sharply differentiated from one another on the basis of the pH of their acid agglutination, but this has not proved to be true.⁵³

⁵³ For a summary of the literature see Gouwens: Jour. Inf. Dis., 1923, 33:113.

are sufficient to induce major changes in the incidence of many infective diseases (Topley 1942; see also McKendrick 1940). Many other factors have been studied in far greater detail but with less control under field conditions. Some day we may be able to work these factors into our immunological picture in terms of the effects produced by changes in the rate at which infection is received, or in the rate at which immunization proceeds (see for instance Dudley 1923); but that day has not come yet.

The Control of Herd Infection. The study of herd infection and herd immunity by the methods described above is as yet in its infancy, but it has already modified many of our conceptions with regard to the administrative control of epidemic disease. Isolation and quarantine, for instance, have, in the past, played a major part in public health administration. A very cursory consideration of the various types of distribution of a bacterial parasite within an infected herd that have been described earlier in this chapter, and of the results obtained in experimental epidemics, will raise serious doubts as to the probable efficacy of such measures. In the case of quarantine, it is clear that it can succeed only where it is complete; and the history of sanitary control suggests that successful quarantine is possible only under very exceptional conditions, and over relatively short periods of time. The carrier and the atypical case defeat a sanitary barrier, just as they defeat isolation; and, under the conditions of modern transit, there seems little possibility of preventing the introduction into any one part of the inhabited world of any infective parasite which is prevalent in another. Once the barrier is passed, the subsequent course of events will depend upon the conditions obtaining within the community into which infection has been introduced.

The policy of the isolation of sick persons within a community is based on a similar failure to realize that the clinical picture provides a very incomplete description of the true state of affairs. If isolation removed from the community the whole, or even the great majority, of the infected individuals, it might be expected to exert a considerable influence on the prevalence of an infective disease. But if the ratio of latent or atypical infections to clinically recognizable cases is high, we cannot hope to effect any marked reduction in the morbidity rate by removing to hospital those cases which exhibit the typical stigmata of the disease. We may indeed effect a smaller reduction in the total infective material than the ratio of isolated to non-isolated, among infected individuals, would suggest; for the sick person would, in any case, move less freely among his fellows than the apparently healthy carrier. As in the case of quarantine, we should expect a policy of isolation to be successful only in exceptional circumstances, when the recognizable cases form a very high proportion of the total infected, and when the total mass of infection to be dealt with is small. Once a given infective disease has assumed an endemic-epidemic prevalence within a herd, we should expect no appreciable result from the isolation hospital, so far as a reduction in morbidity is concerned. It is interesting to find that the expectations based on bacteriological and experimental findings are borne out by administrative experience. Thus, an attempt has been made, by an analysis of the available records, to answer the question: Does hospital isolation have any effect upon the incidence of scarlet fever? All applications of the calculus of correlations have wholly failed to bring out any connection whatever between the incidence rate of scarlet fever and the extent of isolation (see Greenwood and Topley 1925). The value of the isolation hospital must apparently be judged by the benefit which it confers on the sick within its walls; for it would

The agglutination of bacterial cells is, however, a function not only of electrical charge on the cells but also of the cohesive forces tending to draw them together. The studies of Northrup and DeKruif⁵⁴ in which both potential difference (between the cells and the suspending medium) and cohesive force were directly measured have shed considerable light on the mechanism of agglutination. These workers found that electrolytes in low concentration (0.01 N) affect primarily the potential, and in high concentration also decrease the cohesive force. If the cohesive force is not affected, agglutination occurs when the potential is reduced below the critical point of 15 millivolts. If, however, the cohesive force is decreased, the critical potential is also decreased and, therefore, in concentrated salt solutions agglutination does not take place even though there is no measurable potential. Immune serum, presumably adsorbed on the surface of the bacterial cells, while reducing the charge somewhat, appears to function by preventing the salt from decreasing the cohesive force, and agglutination occurs at the critical point of 15 millivolts. Furthermore, if the potential difference is reduced by electrolyte to 15 millivolts or less in a bacterial suspension, the addition of immune serum raises the reduced cohesive force and agglutination takes place. Examination of typhoid bacilli by the electron microscope in the presence of immune serum has shown that the flagella become thickened by the deposition of an antibody film approximately 21 Å thick and the cell walls become more opaque and less definite in outline. The serum-sensitized surfaces appear to be sticky, not only for one another, but also for other particulate matter.⁵⁵

The presence of antigenic cell components may interfere with or prevent agglutination of bacteria by homologous antiserum. The presence of Vi antigen (p. 451) in typhoid bacilli, for example, renders them inagglutinable with homologous O antiserum, but they become agglutinable as the interfering antigen disappears during successive subcultures. Similarly, colon bacilli contain a thermolabile component of the envelope antigen (p. 424), the L antigen, which inhibits agglutination with O antiserum, but the bacterial suspension becomes agglutinable when this component is destroyed by boiling. Possibly the blocking effect is physical in nature in that the O antigen is covered, though it would seem that the forces operative in antigen-antibody union would be effective over the distance imposed by a monomolecular layer of immune globulin.

Precipitins. The mixture of an immune serum prepared against a soluble antigen, such as egg albumin, with its homologous antigen results in the formation of a precipitate. This phenomenon is termed the *precipitin reaction* and the antibody a *precipitin*; the antigen is sometimes designated as a *precipitinogen*. The physical state of the antigen used for immunization is not of particular importance; antibacterial sera, for example, will give precipitates when mixed with preparations of the soluble cell substance of the microorganisms, and precipitins may almost always be demonstrated in lytic, antitoxic, opsonic and agglutinating antisera. It has been found in the first instance that the precipitate will fix complement and the flocculation of toxin antitoxin mixtures has been referred to above. Like agglutination, precipitation does not occur in

⁵⁴ Northrup and DeKruif *Jour. Gen. Physiol.*, 1922, 4:639, 655.

⁵⁵ Mudd and Anderson *Jour. Immunol.*, 1941, 42:251

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the absence of electrolyte and does not require the presence of heat-labile substances such as complement.

In contrast to the agglutination test, the precipitin test is carried out with undiluted, or only slightly diluted, serum but the antigen solution is diluted in series. The reagents may be mixed in the usual way, or the antiserum may be pipetted into very small test tubes and the solutions of diluted antigen carefully layered on top with no mixing. In the first instance a precipitate is formed which settles out like agglutinated bacteria, while in the second a precipitate forms at the interface between serum and antigen solution. The latter test is termed a "ring test." The end point of either test may be taken as the highest antigen dilution with which the serum forms an observable precipitate and the titer of the serum given in these terms. Potent antisera may have extremely high titers, precipitating antigen in dilutions of 1:100,000 to 1:5,000,000. A more accurate measure of the precipitin content of an antiserum, however, is that of the antigen dilution in which precipitation first occurs (and generally is heaviest), for it is at this point, sometimes termed the *equivalence zone*, that the antigen-antibody ratio is optimal for precipitation. Two such optimal ratios may be distinguished, one when the antigen is held constant and the antibody added in increasing amounts and the other when the antibody is constant and antigen is added. The two are not necessarily identical, as might be expected, although very nearly so in the Ramon flocculation, in which the end point is clearly that of optimum antigen-antibody ratio. In other instances the amount of antibody required for most rapid flocculation when the antigen is kept constant may be six to eight times as much as when the antibody is kept constant. The explanation of this phenomenon lies in the variable proportions of antigen and antibody which make up the precipitate, as will appear in a later section.

The precipitate formed consists of antigen and antibody in variable proportions depending upon the relative amounts of the reacting substances. The antigen is diluted rather than the antiserum because relatively large amounts of antibody are required. As in the agglutination reaction, antibody is adsorbed on to the surface of the antigen, but, since the antigen molecules are much smaller than the bacterial cells, the surface to be covered is proportionally greater and consequently large amounts of antibody are required. It follows, of course, that the proportion of antibody to antigen is much greater in the precipitin reaction than in agglutinated bacteria.⁵⁶ The mechanism of the precipitin reaction is probably essentially that of agglutination, molecules instead of bacteria being clumped. It will be discussed at greater length in a later section.

The close relation between the precipitation and agglutination reactions is indicated by the agglutination by a precipitating antiserum of particles of inert material, such as collodion, upon which the antigen has been adsorbed. These particles then behave as particles of antigen and may be set up with serial dilutions of antiserum as in the agglutination test.⁵⁷ Goodner⁵⁸ has carried out the reaction in the reverse by adding collodion particles to the antigen-antibody

⁵⁶ Cf. Zinsser: Jour. Immunol., 1930, 18:483.

⁵⁷ Cannon and Marshall: Jour. Immunol., 1940, 38:365.

⁵⁸ Goodner: Science, 1941, 94:241.

another group the organisms form a felted mycelium without any radial arrangement, and without any formation of granules. Some authors prefer to limit the term actinomycosis to diseases of the former group, denoting those of the latter group by such terms as Streptothricosis, Cladothricosis, Oosporosis, Pseudotuberculosis, Para-actinomycosis, or Pseudo-actinomycosis. In Chapter 14 we have given our reasons for including all the filamentous branching organisms in the *Actinomyces* group. The logical consequence of this is to classify all diseases caused by these organisms under the name of Actinomycosis. This term, however, is often used in a broad sense to include chronic granulomata characterized by club formation. Such granulomata are in fact sometimes caused by *Staph. aureus* or *C. pyogenes*, and though their inclusion may be justified on clinical grounds, it leads to great confusion bacteriologically. We therefore propose to exclude from Actinomycosis the granulomatous lesions of cattle and swine that are caused by organisms other than those belonging to the *Actinomyces* group. The staphylococcal lesions are better referred to as Botriomycosis (see Chapter 67).

ACTINOMYCOSIS.

- A. Due to *Actinomyces bovis* of Wolff and Israël. True ray-fungus disease in man and cattle. Granules or *Drusen* formed in tissues. Anaerobic.
- B. Due to *Actinomyces*—aerobic types.
 - (1) Non-acid-fast group. Madura foot—granules found in tissues. Other lesions in man and animals—no granules found in tissues.
 - (2) Acid-fast group—chiefly pulmonary and abdominal infections in man. No granules formed in tissues. Farcy of cattle—no granules formed in tissues.

ACTINOBACILLOSIS.

Due to bacilli.

- (1) *Actinobacillus lignieresii* of Lignières and Spitz. Actinobacillosis in cattle. Granules or *Drusen* formed in tissues.
- (2) *Actinobacillus actinoides* of Theobald Smith. Broncho-pneumonia of calves: pneumonia of rats. No granules formed in tissues.
- (3) *Actinobacillus muris* Rat-bite fever (one type) in man, and infective arthritis in mice. No granules formed in tissues.

ACTINOMYCOSIS

A. Due to *Actinomyces bovis* Wolff and Israël

Epidemiology.—Actinomycosis in man is not a common disease. According to van der Hoeden's (1939) observations in the Netherlands, it is three times as common in males as in females, and ten times as common in rural districts as in large towns; about 80 per cent. of the cases occur in patients over 20 years of age. The cervico-facial region is the commonest situation attacked. Table 90, compiled from cases observed by Illich and by Leith (see Delépine 1915), and Colebrook (1921), indicates the distribution of the lesions. Cope (1938) collected a rather larger series of cases in his monograph on actinomycosis, but the percentage distribution is much the same.

The fatality of the disease depends largely on the site affected. Of 10 cases of cervico-facial infection observed by Colebrook (1921), 9 recovered: on the

mixture, the complex being adsorbed and the particles agglutinated, he has called this "collodion fixation."

The precipitin reaction occurs not only between antibody and complete antigen but also with some partial antigens or haptenes. An antipneumococcus serum, for example, will react not only with a solution of the pneumococcus cell substance but also with pure capsular polysaccharide. When the hapten is of high molecular weight, as in the case of the polysaccharides, a visible precipitate is formed, but when it is a simple compound such as an organic acid or monosaccharide no observable precipitate appears. The reaction between antibody and such haptenes may, however, be shown by the fact that antisera treated with hapten will no longer precipitate the complete antigen, the antibody has already reacted with the partial antigen. The development of knowledge of the haptenes has made possible quantitative studies on the antigen-antibody reactions, when both reacting substances are proteins the two cannot be distinguished by chemical methods in a precipitate, but if the one be a polysaccharide and the other a protein, the reacting substances are readily differentiated and determined by ordinary analytical methods.

The precipitin reaction, like the other immunological reactions, is highly specific, but cross reactions are observed between chemically similar antigens that are analogous to "group agglutination." Such cross reactions occur between antisera for the blood proteins of man, chimpanzees, gorillas and various species of monkeys. An antihuman serum prepared by immunizing a monkey, however, does not show cross reaction with that monkey's serum. Other immunological reactions such as those between the sera of various animal species also occur. The use of the precipitin reaction also in forensic medicine has resulted in its frequent use in forensic medicine. The differentiation between chicken blood and human blood by the precipitin test in a case of suspected murder, for example, has the highest standing as evidence in court.

The studies of Landsteiner and others on the chemical basis of the specificity of the precipitin reaction have resulted in its frequent use in forensic medicine. The differentiation between chicken blood and human blood by the precipitin test in a case of suspected murder, for example, has the highest standing as evidence in court.

Ablastin. The existence of an antibody which inhibits the reproduction of an invading microorganism was postulated by Ascoli⁵⁵ on the basis of his experiments with the anthrax bacillus. Similar results were reported later by Dochez and Avery⁵⁶ for the pneumococcus which suggested that immune serum temporarily inhibited the multiplication of these organisms and depressed their proteolytic and glycolytic activities. This effect was attributed to "antiblastic" or reproduction inhibiting antibodies present in the immune serum. In reported experiments with bacteria, however, the results may be interpreted in other ways, and the existence of an abl原因 for bacteria has not been conclusively demonstrated.

⁵⁵ Ascoli: *Ztschr. f. physiol. Chem.*, 1906, 45-220, *Centralbl. f. Bakt.*, Abt. II, 1909, 46-178.

⁵⁶ Dochez and Avery: *Jour. Exp. Med.*, 1916, 23-61.

view appeared probable because the organism was found to lead a saprophytic existence outside the body, being frequently isolated from grasses and cereals, and because awns of grasses, or barley spikes, are not uncommonly found in the actual lesions. But now that Bostroem's organism is regarded as an accidental contaminant, and the organism isolated by Wolff and Israël as the true cause of the disease, this hypothesis has been abandoned. Wolff and Israël's organism appears to be a strict parasite; it has never been found outside the animal body, and its survival in artificial culture is brief. These facts suggest that the disease is transmitted by infected pus, saliva, or nasal discharge.

Strong evidence in favour of this view is provided by the work of Naeslund (1925, 1926, 1929) who, in a study of the flora of the human mouth, succeeded in isolating both aerobic and anaerobic types of *Actinomyces*. The anaerobic types were indistinguishable from *Actinomyces bovis*, and were more frequently met with



FIG. 272.

Part of the edge of a colony of *Actinomyces bovis* in the jaw of an ox, showing the peripheral zone of clubs ($\times 350$).

than the aerobic types. They were, moreover, isolated from a high proportion of salivary calculi, thus supporting Söderlund's (1921) view that the formation of these calculi must be regarded as a specific result of actinomycotic infection. The same organisms also seemed to play an important part in the development of dental tartar. Bibby and Knighton (1911) regard Naeslund's micro-aerophilic and anaerobic strains as probably belonging to the *Leptothrix* group; but the work of Lord and Trevett (1936), Emmons (1938), Sullivan and Goldsworthy (1940), and Slack (1912) seems to leave little doubt of the occurrence of true *Actinomyces* strains, indistinguishable from the Wolff-Israël type, in the human mouth, notably in diseased tonsils, carious teeth, and pyorrhoeal pus. If this organism is accepted as an inhabitant of the human mouth, the factors predisposing to the development of actinomycosis remain to be determined. Possibly suitable necrotic foci, such as septic teeth, have to be present in which the organisms can develop under anaerobic conditions, or other organisms may be necessary to favour its growth (Holm 1950). There are records of three cases in which the disease followed a human bite (see Robinson 1944).

Bacteriology.—In the tissues *Actinomyces* grows in the form of colonies or *Drusen*, which macroscopically have the appearance of small granules. A section suitably stained reveals the presence of a central mass of partly necrotic material, in which branching filaments may be recognized, and a peripheral zone of swollen bodies looking like clubs and arranged radially. The clubs are surrounded by pus cells, outside of which there are aggregations of mononuclear cells and dense masses of fibrous tissue. The colony

A reproduction-inhibiting antibody developed in response to invasion with certain of the parasitic protozoa, trypanosomes, was independently discovered by Taliaferro.⁶¹ In this case the activity of the antibody was sharply differentiated from that of coexisting lysin and was demonstrable directly by cessation of cell division and indirectly in terms of variability in size of the microorganisms, i.e., in a population of rapidly reproducing organisms the size of the individual cells is subject to wide variation whereas size is relatively constant in a population consisting entirely of adult forms. It is not necessary, however, to postulate the existence of an antibody which specifically inhibits reproduction, for the consequences of the union of antibody with the antigen of the living microorganism may well be such as to inhibit normal physiological functions including those associated with growth and cell division. For example, *Nippostrongylus* larvae in and about which immune precipitates have been formed are stunted and immobilized.

Perhaps related to ablastins are the "non-absorbable" antibodies which have been demonstrated in certain worm infections. Campbell⁶² has found that antibodies to the larval tapeworm, *Cysticercus crassicolis*, present during the later stages of infection, cannot be absorbed from the serum by treatment with the parasites. He regarded these nonabsorbable protective substances as anti-enzymes, whose presence interferes with the processes of growth and reproduction, but which are not absorbed because the antigen is elaborated only by actively metabolizing cells and is not present in those which are physiologically quiescent.

Possibly to be regarded in the same light is the precipitin present in convalescent yellow fever serum which reacts with yellow fever serum taken during the acute stage of the disease but not with the virus.⁶³ This phenomenon, however, has been interpreted by some as indicative of the presence of a "pathological protein" in yellow fever. Avery and his co-workers⁶⁴ have observed a similar protein which appeared in the blood of human beings and of monkeys in various etiologically unrelated infections, and which formed a precipitate with the C substance (somatic polysaccharide) of pneumococci. Serum showing this activity has been called "acute phase serum." The C-reactive protein has been separated from serum proteins in crystalline form by McCarty⁶⁵ and shown to be immunologically specific.

Neutralizing Antibodies. In the light of the capacity of an immune serum to lyse, kill or sensitize bacteria to phagocytosis, it follows that the injection of a suspension of pathogenic bacteria in homologous immune serum may have less serious consequences to the susceptible animal than the inoculation of the bacteria alone. Since the usual serological reactions may be carried out with the filterable viruses only at considerable inconvenience owing to difficulties in the preparation of the antigen and the like (p. 853), it has become customary to evaluate the antibody content of antiviral sera in terms of

⁶¹ Cf. Taliaferro Amer. Jour. Hyg., 1932, 16:32.

⁶² Campbell: Jour. Immunol., 1938, 35:195, 205, 465. Jour. Inf. Dis., 1939, 65:12.

⁶³ Hughes. Jour. Immunol., 1933, 25:275.

⁶⁴ Avery, Abernathy and MacLeod: Jour. Exp. Med., 1941, 73:173 et seq. See also Lofstrom. Brit. Jour. Exp. Path., 1944, 25:21, Lofstrom: Acta Med. Scand., 1943, Suppl. 141:1, Hedlune. *ibid.*, 1947, Suppl. 196:596.

⁶⁵ McCarty: Jour. Exp. Med., 1947, 85:491.

Magnusson found that, though the disease could not be satisfactorily reproduced in small animals, it was possible to set up typical actinomycotic lesions by the inoculation of pure cultures into cattle. That it has not been cultured from all cases of the disease is probably explained by technical difficulties, especially when the lesion is invaded by secondary organisms, and by the not infrequent sterility of granules from old lesions.

We may conclude that the evidence in favour of the ætiological rôle of the *Actinomyces bovis* described by Wolff and Israël is sufficient to justify us in accepting this organism as the cause of that variety of actinomycosis which is characterized by the presence in the tissues of the typical ray fungus.

It may not, however, be the only member of the *Actinomyces* group that is capable of giving rise to the disease. Holm (1950) for example, found that of 219 anaerobic strains cultivated from human cases only 122 conformed to the Wolff-Israël species; the remaining 97 strains fell chiefly into 3 different species, which have not yet been fully studied. Holm lays great stress on the invariable accompaniment of *Actinomyces bovis* and these newly recognized species by other organisms—mainly anaerobic bacilli and cocci; of these, one of the commonest is the organism described by Klinger in 1912 as *B. actinomycetem comitans*, and subsequently noted by Colebrook (1920) and Bayne-Jones (1925) (see p. 473). Holm postulates that actinomycosis is caused by *Actinomyces bovis* acting not alone, but in synergism with other anaerobic organisms. It should be added that *Actinobacillus actinomycetem-comitans* is occasionally isolated in pure culture from closed lesions (Goldsworthy 1938, Thjøtta and Sydnæs 1951).

Diagnosis, Prophylaxis and Treatment.—Diagnosis is made by microscopical and cultural examination of the affected tissues. The granules should be crushed between slides and stained by Gram's method. If calcified, they should first be treated with hydrochloric acid. The presence of a mycelium of Gram-positive filaments surrounded by radially disposed Gram-negative clubs is characteristic of actinomycosis. Cultures are made from fresh young granules only; after thorough washing, they are seeded into a number of tubes containing blood broth (Gordon 1920), glucose agar, or serum agar; incubation is carried out under both aerobic and anaerobic conditions, preferably in an atmosphere to which 5–10 per cent. CO₂ has been added. Rosebury, Epps and Clark (1944) prefer to streak the unwashed granule or the pus on to four plates in series of Bacto brain heart infusion agar and to incubate in an anaerobic atmosphere to which 5 per cent. CO₂ is added. Primary cultures are frequently contaminated; single colonies should therefore be picked off to obtain pure cultures. Examination of the agglutinating power of the patient's serum to the organism isolated should be carried out if possible. In pulmonary actinomycosis in man the sputum is tough, often viscous, and sometimes hæmorrhagic; the typical granules can be found on examination (Harbitz and Gröndahl 1911). Most of the abdominal cases commence in the appendix. In the rare cases of actinomycotic meningitis, the primary lesions are generally in the lungs and bronchial glands (Henry 1910).

As we are still ignorant of the factors determining the development of the disease, it is difficult to lay down prophylactic measures against it. It is clear, however, that the discharge from the lesions should be considered dangerous, and every care taken to prevent its coming into contact with man or other animals.

Though vaccine therapy was reported on favourably in the past (Colebrook 1921, Negróni 1937), treatment of the disease is now carried out mainly with

their neutralizing capacities, i.e., ability to prevent infection when injected with the infectious material. This technique is, however, not confined to the filterable viruses, for the "protective titers" of certain antibacterial sera, as, for example, in studies on the efficacy of typhoid immunization (p. 461), have been found a more satisfactory measure of antibody content than the measurement of agglutinin, precipitin or other single antibody titers. It is becoming more and more generally used as a method of assay of the potency of antisera.

In practice, varying amounts of immune serum are mixed with a constant amount of bacterial suspension or infectious material and injected into susceptible animals either with or without a period of preliminary incubation. In this way a crude approximation of the neutralizing or protective capacity of an immune serum can be made.

Whether the "virus neutralizing" or "protective" capacity of an immune serum can be accounted for on the basis of the combined activity of the other known antibodies or whether other factors are involved is open to question. Since, however, the differentiation of the kinds of antibodies from one another is largely a matter of the technique by which the antigen-antibody reaction is demonstrated, as will appear, the neutralizing or protective action of an immune serum which is not identical with precipitin, agglutinin or other antibody is legitimately regarded as a separate antibody.

The Nature of Antibodies. The question of the nature of the antibodies is one of no small practical as well as theoretical importance. These substances are intimately associated, if not identical, with the globulin fraction of the serum proteins and may be separated from the other serum constituents, sometimes by simple dilution with distilled water but more commonly by some salting out procedure. With one or two exceptions, the range of salt concentration over which they are precipitated is broad and not sharply defined. Attempts to further differentiate the active fraction into euglobulin and pseudoglobulin have not been successful, the antibody appearing sometimes in the one, some times in the other and not infrequently in both, depending upon the animal immunized. Electrophoretic fraction of serum globulin in the Tiselius apparatus, however, allows the separation of three components on the basis of mobility. These are designated α -globulin, β -globulin and γ -globulin in order of decreasing mobility. Antibody appears to be associated almost exclusively with the γ -globulin fraction, though β -globulin may occasionally show some activity. Probably the purest antibody preparations have been those obtained by dissociating antigen-antibody precipitates, the antibody having been previously purified by salting out; about 0.01 mg. of globulin per unit of diphtheria antitoxin is precipitated by toxin. Northrup⁶⁴ has prepared crystalline diphtheria antitoxin which appears to be a pure protein, crystallizing in thin plates and containing 700,000 to 1,000,000 antitoxic units per gram. It has not been possible as yet, however, to separate antibody from serum globulin and, in spite of older reports of the preparation of non protein antibody solutions, present evidence justifies the current belief that *antibodies are modified serum globulin*. The physical and chemical properties of antibodies are, then, those of globulin. More precise knowledge is obviously directly dependent upon the state of knowledge of protein chemistry, whose present unsatisfactory nature

⁶⁴ Northrup *Proc. Amer. Phil. Soc.*, 1941, 85 13.

Henrici and Gardner (1921) isolated a similar organism from the sputum of a woman, which differed, however, in certain particulars, and which they called *Actinomyces gypsoides*. From the literature they were able to gather accounts of 26 cases of infection with acid-fast *Actinomyces*. Infection probably occurs by inhalation; the first lesions are in the peribronchial nodes. Later, caseous broncho-pneumonia develops, followed by central softening and cavity formation. Metastases may occur. The duration of the illness is generally about 6 months. These cases are liable to be confused with tuberculosis. Goldsworthy (1937) recommends that the organisms should be sought for in the sputum of all patients with obscure or unusual chronic infections of the lungs, especially when repeated attempts to demonstrate the tubercle bacillus have failed. The organisms are liable to be decolorized in the ordinary Ziehl-Neelsen process, but their filamentous morphology is rendered strikingly evident by Gram's stain. Cultivation of the organism, and the exclusion of tuberculosis by inoculation of the sputum into guinea-pigs, should effect a differential diagnosis. Penicillin appears to be of value in treatment.

Actinomyces of the aerobic non-acid-fast type have been described in various lesions by numerous authors, such as in the bladder and prostate of a man with pyuria by Cohn (1913), in the purulent cornea by Namyslowski (1912), in the cerebro-spinal fluid of children with meningitis by Gerbası (1927), and in fatal infections of the lungs by Biggart (1934) and Lynch and Holt (1945).

(3) Cattle Farcy. Farcin du bœuf.

Cattle farcy is characterized by the appearance—generally on the medial surface of the extremities—of firm, painless perivascular nodes, which later suppurate. When incised, they discharge a whitish, odourless mass resembling soft cheese (Report 1913). The regional lymph glands become converted into firm, painful tumours. It is a chronic disease lasting for a year or more, and marked in the later stages by severe cachexia. From the pus of an affected animal Nocard (1888) isolated an aerobic branching organism, now known as *Actinomyces farcinicus*. Intravenous injection of pure cultures into a cow and a sheep resulted in the development of milary nodules. The disease is not common in Europe; it is very prevalent in Guadeloupe, and has been recorded in India, and in Kenya (Daubney 1927).

(4) Other Lesions in Cattle.

Evans (1918) has recorded an infection of the udder of cows with an organism of the aerobic *Actinomyces* group. She succeeded in isolating it from 18 out of 21 samples of milk, in which it was present in considerable numbers.

(5) Goats, Horses and Dogs.

Silberschmidt (1899) isolated an organism of this group from three goats; this organism is called *Actinomyces capræ*. The same organism was later cultivated by Galli-Valerio from the lung of a goat. Dean (1900) cultivated a member of the group from an abscess in the submaxillary region of a horse. Gunsberg and Little (1948) described two cases in dogs. One was caused by *Actinomyces asteroides* and affected the mouth. the other was caused by a non-acid-fast organism and affected the abdomen.

sera may, then, appear without invalidating the concept of the unity of antibodies; the flagellar agglutinins for the typhoid bacillus, for example, may have quite a different titer from that of precipitins for the cell substance. Similar discrepancies in complement-fixing and precipitin titers of antisera for pure antigens, such as egg albumin, in which only a single antibody is concerned, are explained by the fact that antigen-antibody ratios optimal for complement fixation are not optimal for precipitation.

The evidence for the essential identity of what were at first regarded as distinct antibodies for a single antigen is indeed impressive, and the unitarian hypothesis may be regarded as substantially in accord with the facts.

THE ANTIGEN-ANTIBODY REACTION

The mechanism of the antigen-antibody reaction has already been touched upon briefly in the preceding sections. The reaction between these two substances may be considered here, however, at somewhat more length and in general terms.

It will already be apparent that the antigen-antibody reaction appears to take place in two separate and distinct stages, the first in which the two combine and the second in which the consequences of that union appear as agglutination, precipitation and the like. In mixtures the two processes undoubtedly go on at the same time; it is not necessary that the first proceed to completion before the second may be initiated. Direct evidence of such a two-step reaction is found in the union of antibody with simple haptenes, in which the former is saturated and unable to react further with complete antigen even though no second stage is observable. The theoretical aspects of this union are concerned not only with the actual mechanism of union but also with the attributes of antigen and antibody that make possible their reaction with one another.

The only comprehensive formulation of the theoretical aspects of immunology is that of Ehrlich,⁷³ a formulation which has constituted the theoretical basis upon which a vast amount of experimental work has rested. Let us consider Ehrlich's basic concept, first as it was developed, and second in the light of present knowledge.

Ehrlich's Receptor Theory. Ehrlich's attempt to develop a theoretical basis for the explanation of the immunological phenomena has been touched upon briefly in connection with the antitoxins. The theory he developed, however, is not confined to the toxin-antitoxin reaction but is a general one of immunity in the broad sense.

The receptor theory rests upon the basic assumption that the various cells of the animal body obtain their nutriment from the blood or lymph in which they are bathed through the agency of localized cell substances, the *cell receptors*, which have combining affinities with food substances. The receptors may be regarded as bearing the same relation to the main body of the cell (*Leistungskern*) that the side chains of complex molecules bear to the central molecular nucleus; hence the receptor theory is sometimes known as the *side chain theory*. These receptors may be of simple constitution, adapted to the taking up of relatively simple substances, or they may be highly complex and capable of

⁷³ Ehrlich *Studies on Immunity*. New York. 1906.

Gram-negative, non-motile, cocco-bacillus, which Lignières and Spitz called the actinobacillus—now called *Actinobacillus lignieresii*. (For description see Chapter 14.) In this country the disease is less acute, and the lesions are more localized, though the glands are almost invariably affected.

Reproduction of the Disease in Animals.—Subcutaneous inoculation of a pure culture into cattle leads to the formation of an abscess similar to that occurring in the natural disease, though not all strains are virulent. Fluctuation is apparent about the 10th day. The abscess enlarges, and eventually the skin gives way, allowing a small quantity of pus to exude. Fungating masses spring up around the opening and obstruct drainage. The animals gradually waste. The pus contains typical granules. Feeding has not been successful in transmitting the disease. A local abscess develops after subcutaneous inoculation of pure cultures into pigs and sheep. In horses a large œdematous swelling is formed, which discharges pus after a few days.

The work of Lignières and Spitz has been confirmed by workers in several countries. Nocard (1902) recognized the disease in France. He pointed out that in actinomycosis of cattle the granules in the pus are yellowish in colour and often calcified, whereas in actinobacillosis they are greyish-white and rarely calcified. Griffith (1916) examined the diseased tongues and lymphatic glands of 44 cattle slaughtered in this country. Of these no fewer than 40 had lesions of actinobacillosis. From 23 of these he isolated a Gram-negative bacillus which had the characters of *Actinobacillus lignieresii*. One of the cultures inoculated subcutaneously into a calf caused a local lesion containing typical granules. The remaining 4 cases, affecting the lower jaw, were due to the *Actinomyces bovis*. In an examination of 34 specimens of "actinomycosis" in cattle slaughtered at Islington, Bosworth (1923) recognized *Actinobacillus lignieresii* 21 times; from 17 of the cases he isolated it in pure culture. The other 13 specimens were examples of actinomycosis.

Diagnosis.—The important points are: (1) The location: practically all jaw lesions appear to be due to *Actinomyces bovis*, whereas lesions of the soft parts—tongue, cheek, gum, palatal mucosa, skin, and lymphatic glands—are due to the actinobacillus. (2) The frequent involvement of the lymph glands in actinobacillosis; in actinomycosis the glands are swollen only as the result of secondary infection from open lesions. (3) Granules are rarely found in the glands in actinomycosis, very frequently in actinobacillosis. (4) In actinobacillosis the granules are paler in colour, and rarely calcified, in actinomycosis the granules are darker in colour and often calcified. (5) In actinobacillosis the granules consist of a central mass of detritus containing minute Gram-negative bacilli surrounded by long, radially disposed clubs; in actinomycosis recently formed granules contain a central Gram-positive mycelium, together with Gram-positive rods and coccoid forms, surrounded by short, radially disposed clubs; older granules may show a few Gram-positive bacillary and granular elements embedded in a Gram-negative matrix. (6) In actinobacillosis cultures reveal the presence of a small Gram-negative, aerobic cocco-bacillus; in actinomycosis a Gram-positive, branching, filamentous organism, often assuming rod forms in culture, not unlike *C. diphtheriæ*, is isolated under anaerobic conditions. During life the agglutination reaction may be of value. The serum of animals suffering from actinobacillosis frequently agglutinates the causative organism to a titre of 1/50 or over.

anchoring large and complex protein molecules. Each cell may, of course, contain a large number of receptors of different affinities and degrees of complexity.

It is plausible to assume that when bacteria or other alien cells or their products are introduced into the body, the combining affinities of certain receptors may be satisfied by bacterial substances just as by similarly constituted food molecules. The anchoring of toxic substances, however, unlike that of food substances, is followed by damage to the cell and loss of the particular side chain or receptor that unites with the toxic element. When injury to the main body of the cell is not carried too far, repair can take place and the receptors be regenerated. There is a tendency in the regeneration of lost parts, in this case receptors, to over-compensate, and the free receptors formed in excess of the needs of the cell are discharged into the blood stream. *These free receptors are the antibodies*, the antitoxins, agglutinins, lysins and the like. They differ in complexity. The simplest, or receptors of the first order, are those which combine with toxin and, when free, constitute antitoxin, receptors of the second order are somewhat more complex and are functional in agglutination and precipitation, receptors of the third order are still more complex and function in the lytic reactions in which complement plays a part.

Similar representations of the antigenic substances and of complement necessarily follow in this concept of the nature of the immune bodies. Toxin, for example, is assumed to be a relatively simple substance with two functional parts, a haptophore which unites with the receptor and a toxophore which exerts the poisonous effect of the substance. In this terminology, toxoid would be regarded as toxin in which the toxophore portion is inactivated or destroyed.

? The mechanism of the reaction of the antibodies with their respective antigens is apparent from the foregoing considerations. The union of toxin and antitoxin is the simplest type, the haptophore group uniting specifically with the corresponding portion of the free receptor or antitoxin molecule and the toxophore group being neutralized in that it is prevented from exerting its action on the cell. Agglutination is much the same so far as the union of antigen and antibody is concerned, but the presence of the zymophore group brings about clumping. In the case of complement fixation it is assumed that the receptor of the third order, after breaking off, functions as a sort of bridge or intermediary body (*Zwischenkörper*) which unites with complement at one end and with antigen at the other, thus sensitizing the antigen to the lytic action of complement.

It will be clear that, in its simplest terms, the receptor theory is a plausible explanation of immunological phenomena. There is evidence, for example, that the various bacterial toxins are bound in each case to particular cells of the organism. The tetanus toxin, when mixed *in vitro* with emulsions of fresh organs, manifests an affinity for different organs in different animals. In man, the horse and the guinea pig, only the central nervous system is able to bind the toxin, a finding in complete accord with the pathology of tetanus. If a mixture of tetanus toxin and guinea pig brain emulsion in suitable proportions is injected into a susceptible animal, the animal is entirely unaffected, just as if tetanus antitoxin (free receptors) had been used in place of fresh cell substance (cell receptors). Other phenomena are equally well explained in terms

which they gave the name *Streptobacillus moniliformis*. It was isolated from the blood of a laboratory worker who was suffering from an acute disease characterized by fever, sore throat, papular erythema, and multiple arthritis. The following year Parker and Hudson (1926) gave an account of an epidemic disease occurring in Haverhill, Massachusetts. This disease they referred to as Erythema arthriticum epidemicum or Haverhill fever. An organism was isolated—*Haverhillia muris formis*—which was subsequently proved to be identical with *Streptobacillus moniliformis*. In neither instance was there any suspicion of infection having occurred through rats. In 1933 Strangeways accidentally encountered the same organism in mice that had died after intraperitoneal inoculation with the blood of rats infected with *Trypanosoma equiperdum*. Investigation showed her that *Streptobacillus moniliformis* was an inhabitant of the nasopharynx of the rat, in which it normally led an apparently harmless existence. A very similar organism had already been reported by Tunncliffe (1916) in the lungs of white rats affected with bronchopneumonia. It would therefore appear that *Streptobacillus moniliformis* is no other than the *Streptothrix muris ratti* of the earlier workers. Both organisms appear to be closely related to the *Actinobacillus* group, and we have therefore suggested for them the name *Actinobacillus muris*. Since this organism is pathogenic for man and is a normal parasite of the rat, it is not difficult to understand how it may be responsible for human infection. *Spirillum minus* is also a normal parasite of the rat, and may likewise cause rat-bite fever. We shall restrict ourselves at the moment to the description of the type of disease caused by *Actinobacillus muris*. For the type caused by *Spirillum minus* see p. 2056.

Rat-bite fever due to *Actinobacillus muris* is an irregularly relapsing, febrile septicæmic disease characterized by metastatic arthritis and morbilliform and petechial cutaneous eruptions. The incubation period, as a rule, is under 10 days, and may be as short as 3 days. The disease is often ushered in by a chill and a rapid rise of temperature. The wound usually heals without exacerbation. There is extreme prostration, severe generalized muscular pain and tenderness, headache, weakness, and a widespread morbilliform or petechial eruption. Generalized enlargement of the lymph glands may occur. Non-suppurative arthritis affecting one joint after another, is a characteristic feature of the disease. There is a leucocytosis with a high proportion of neutrophils. After a few days the temperature falls by crisis, and the disease then assumes a relapsing type, febrile paroxysms occurring at irregular intervals for weeks or months. The case fatal or not is not known with certainty, but is probably round about 10 per cent. Little is known of the post-mortem appearances, but Blake's case showed an ulcerative endocarditis and a subacute myocarditis. Diagnosis during life is best made by cultivation of the causative organism from the blood stream during febrile attack. About 20 ml. of blood are distributed partly into serum broth and a number of plates of Loeffler's serum. The cultures may be incubated aerobically or anaerobically, preferably in the dark, at 37°C. of 5 per cent. CO₂. Brown and Nunemaker (1942) found *Act. muris* in blood serum in a titre varying from 1/5,120 to 1/1,000,000. It may be prepared by formalizing the serum with 1 per cent. formalin. *Act. muris* that forms a typical colony on Loeffler's serum, but its real value is not known. Observations on mice, it is without doubt, are against rapidly fatal infection.

of this theory. It will be recalled, for example, that complement was early separated into two parts, mid-piece and end-piece, a finding in keeping with the postulation of zymophore and haptophore portions of this substance.

Certain inadequacies of the receptor theory were apparent almost from the beginning, however, since new knowledge, rather than being predicted by the theory, necessitated various modifications. These discrepancies arose, for the most part, in connection with the mechanism of the first stage of the antigen-antibody reaction and, to some extent, with that of the second stage. It will be recalled that the toxin-antitoxin reaction, for example, was regarded by Ehrlich as analogous to the neutralization of a strong acid by a strong base, a concept that was brought into harmony with experimental observation through the postulation of components of toxin of varying degrees of avidity for antitoxin. Similar modifications and additions, which cannot be considered in detail here, resulted in the course of time in an unwieldy structure that became obviously unsatisfactory.

It may be noted here that the concept of Arrhenius and Madsen of the antigen-antibody reaction as analogous to the neutralization of a weak acid by a weak base was, in essence, an attempt to retain much of the receptor theory and at the same time account for the Danysz phenomenon and related observations as well as the dissociation of antigen-antibody complexes.

The Antigen-Antibody Reaction as an Adsorption Phenomenon. In many respects the antigen and antibody solutions are best regarded as colloidal systems and the union of these substances as adsorption phenomena, physical in nature rather than chemical. This interpretation has been strongly urged by Bordet⁷⁴ and others. Stripped to its essentials, such an interpretation means that the Freundlich isotherm⁷⁵ describes the antigen-antibody reaction with a reasonable degree of accuracy. The successful application of this concept to the first stage of the antigen-antibody reaction is well illustrated in the case of the toxin-antitoxin reaction which has been discussed in this connection in an earlier section. Similarly, the clumping of bacteria under the influence of immune serum, an example of the second stage of the reaction, responds equally well to such an interpretation, as has been pointed out before.

✓The course of events in the antigen-antibody reaction, as seen from this point of view, may be summarized briefly: there is first a specific adsorption of the immune globulin on the surface of the antigen. The cell or protein molecule thus sensitized behaves, not as the unaltered antigen, but as a particle of serum globulin. The aggregation of the coated particles in the precipitin or agglutination reactions is, then, nothing more than the precipitation of this altered colloid by salt. The sensitized antigen is also capable of adsorbing complement whose fixation may or may not be followed by lysis as the nature of the antigen permits. The evidence for some such mechanism is very strong indeed, and it is highly probable that certain phases of the antigen-antibody reactions are essentially manifestations of the properties of colloidal systems. This ines-

⁷⁴ Bordet *L'Immunité* Paris, 1920.

⁷⁵ The relation between the amount of a substance adsorbed and that remaining in solution was given by Freundlich as $x/m = aC^n$, when x is the amount adsorbed, m , the surface on which it is adsorbed, C the final concentration of the substance in solution and a and n are constants.

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capable conclusion led many to abandon the receptor theory as entirely outmoded.⁷⁶

This description of the immune phenomena is by no means entirely satisfactory, however, its most glaring deficiency being its inability to account for specificity. While the union of antigen and antibody is, in all probability, an adsorption reaction, why typhoid antibody is adsorbed on the typhoid bacillus and at the same time not on the dysentery bacillus is unexplained. In addition to this failure to account for immunological specificity, certain minor discrepancies may be observed; there is, for example, agglutination or precipitation over a relatively wide pH range (in the presence of electrolyte) instead of a marked optimum at the isoelectric point of serum globulin, as might be expected. The adsorption isotherm, furthermore, does not approach an asymptote, and the antigen-antibody reaction shows wide variations from expected values at high concentrations of either reagent. These and other minor points are, however, probably of no great significance. In general, it may be said that although this adsorption theory is thoroughly satisfactory in many respects, it accounts only for some, by no means all, of the immunological phenomena.

The Modern Concept. As might be expected, the modern concept of the mechanism of the antigen-antibody reaction is an outgrowth of the theories of Ehrlich and Bordet. The reacting substances are regarded as colloids whose union lies in the realm of surface chemistry and is in part physical and in part chemical. For purely technical reasons, the observations which have led to this concept have been made on the precipitin reaction for the most part, a discussion of the present position is, then, best carried on in terms of this reaction.

It is already apparent from the earlier discussion of antigens that immunological specificity may be conferred by a relatively small prosthetic group or haptene attached to the large protein molecule. Furthermore, it was pointed out that similarities in the structure and configuration of these determinant groups are causally related to the immunological behavior of synthetic antigens. Immunological behavior means, of course, the ability both to provoke the formation of antibodies and to react with them. The significance of these observations on immunological specificity to the nature of the antigen-antibody reaction will be apparent at this point; the molecular structure of the prosthetic group determines whether or not the antigen and antibody will react.

There is good experimental evidence, too detailed and extensive to be considered here,⁷⁷ that the union of antigen and antibody is an adsorption in which a particular molecular structure is adsorbed with a high degree of specificity. It is probable that there is no chemical union (many of the determinant groups are not characterized by chemically active radicals) but that the adsorption is a consequence of the operation of intermolecular forces (secondary valencies) whose specificity lies in the nature of the field of force arising from the arrangement of the atoms within the determinant group. The

⁷⁶ Cf. Manwaring, *Jour. Immunol.*, 1926, 12:177; also a chapter by the same writer in Jordan and Falk, *Newer Knowledge of Bacteriology and Immunology*, University of Chicago Press, Chicago, 1928.

⁷⁷ Cf. Marrack, *The Chemistry of Antigens and Antibodies*, Medical Research Council Special Report Series No. 230, 1939. Also Heidelberger, *Bact. Rev.*, 1939, 3:49.

CHAPTER 53

SWINE ERYSIPELAS, ERYSIPELOID, MOUSE SEPTICÆMIA, AND INFECTIVE MONONUCLEOSIS OF RABBITS

SWINE ERYSIPELAS

INTRODUCTORY

SWINE erysipelas is a disease affecting mainly pigs, and caused by the slender Gram-positive bacillus, *Erysipelothrix rhusiopathiæ*. Adolescent animals seem to be most frequently affected, but it occurs at all ages except during the first 3 months of life, when it is uncommon. The finer breeds of pigs are particularly susceptible. Four clinical types of the disease are described (Craig 1926). (1) The acute or septicæmic form. After an incubation period of 1 to 5 days, illness commences with prostration and high fever, anorexia, thirst, occasional vomiting, and conjunctivitis. Twenty-four hours later bright or dark red patches appear on the skin over the ears, snout, neck, abdomen, and inner sides of the forelegs. The case fatality is about 80 per cent., death occurring usually in 3 or 4 days. (2) The urticarial form, or "the diamonds." This is a mild form accompanied by slight malaise and fever, and characterized by the eruption on the 2nd or 3rd day of well-defined, quadrangular or rhombic patches on the skin of the sides, back, and buttocks; the patches are slightly swollen, deep red or violet in colour, sometimes with a pale centre, from 1-5 cm. in diameter, and may become covered with dry crusts which are afterwards cast off. Recovery occurs in a few days; death is unusual. (3) The chronic or cardiac form. This may follow either of the previous types, or may arise independently. Warty vegetations develop on one of the heart valves, generally the mitral. Death may occur suddenly, or the animals may live for several weeks with symptoms of cardiac insufficiency. (4) The joint or arthritic form. This may follow the urticarial form, accompany the cardiac form, or arise independently. The joints of the limbs become enlarged, owing to a synovitis; the animals are stunted, prefer the recumbent posture, and when induced to get up, walk in a stiff fashion on their toes with the back arched. This form is not usually fatal, but interferes with growth and fattening.

Morbid Anatomy.—In animals dying of the septicæmic form the spleen is enlarged, the lymphatic glands are congested, the gastric and intestinal mucosæ are reddened, and the lungs are œdematous; there are sometimes small hæmorrhages on the serous and mucous membranes and beneath the endocardium. The bacilli are found in small numbers in the blood, spleen, kidneys, and other organs, and in the secretions and excretions. In the cardiac form they are chiefly found in, and may be confined to, the vegetations on the valves.

Epidemiology.—Swine erysipelas is common in this country and throughout Europe, and is met with in the United States of America. It occurs particularly in

IMMUNITY—THE IMMUNE STATE

The foregoing discussion of antigens, antibodies and the antigen-antibody reactions is, essentially, a consideration of the various components of that state of increased specific resistance designated as immunity. The question arises, then, as to what extent these component parts represent the immune state. Can they be put back together to yield a satisfactory account of this phenomenon? In general, it may be said that such a synthesis does result in a relatively complete picture; it is certainly far more satisfactory than corresponding attempts to analyze virulence and non-specific resistance.

It will be clear from preceding considerations that two kinds of activity, closely interrelated, are operative in immunity; the antibodies, present in the body fluids, function in the first case, and the body cells, the phagocytes, in the second. The two are not infrequently separated, the activity of antibodies constituting the subject matter of *humoral immunity* and that of the phagocytic cells making up the subject of *cellular immunity*.

Humoral Immunity. Since the appearance of antibodies follows recovery from many of the infectious diseases, it is tempting to assume that these substances are causally related to the observed state of increased resistance. Observation and experimental evidence have amply justified this assumption, obviously in some instances and in a more subtle fashion in others.

An antibody whose association with the immune state is direct and clear is antitoxin. The toxin produced by the invading microorganism has an affinity for the cells as well as antitoxin present in the body fluids, and the protective effect of circulating antibody must, then, be due in part to its superior avidity for the toxin. Such superior avidity generally, but not always, exists. Instances are known in which the toxin unites with the tissue substance in preference to combining with the antitoxin in the blood. The affinity of the cell substance for the toxin is not a constant quality, but fluctuates under different conditions, notably in the upper limits of active immunization. The tissues of an animal treated with increasing doses of toxin sometimes become hypersensitive to the action of the toxin and, in spite of the fact that large quantities of antitoxin are circulating in the blood, the toxin combines by preference with the tissue substance and causes the death of the animal. Variation in the avidity of circulating antitoxin has been pointed out above. In general, however, the presence of circulating antitoxin is the determining factor; for example, it is not necessary that antibodies to the bacterial cell substance be present in an individual immune to diphtheria or tetanus. The skin tests for immunity, the Schick test for immunity to diphtheria and the Dick test for immunity to scarlet fever, are

per cent. of the animals inoculated. For this reason its use has been very largely abandoned.

To overcome this disadvantage, sero-vaccination was introduced. In Lorenz's method (1893, 1894, 1896), a dose of antiserum is injected, followed after about 4 and 14 days by injections of living virulent bacilli. The serum provides a passive immunity, which is converted by the living vaccines, given subsequently, into an active immunity. In Leclainche's method the living vaccine is mixed with the serum, and the two are injected together. A second injection of vaccine alone is given 10 or 12 days later. Both these methods have been widely used, and appear to give satisfactory results. Both of them suffer from the disadvantage that living bacilli are introduced into the body, with the resultant production of healthy carriers and the risk of spread of infection to other animals.

The method now commonly used is based on the work of Traub (1947) in Germany, who confirmed the observation of a Hungarian worker, Birô, that some strains of *Ery. rhusiopathiæ* form a soluble protective substance in broth culture. This can be adsorbed on to alum and combined with the formalized bacteria. The resultant vaccine, when properly prepared (see Eiszner 1952), gives very satisfactory results in the field. One dose is said to protect pigs for 4-6 months, two doses for 8-12 months (Fluckiger 1952). A dried vaccine is also under trial.

For the treatment of swine that have already developed the disease the injection of 10-30 ml. of antiserum is recommended. Though *Ery. rhusiopathiæ* is sensitive to penicillin *in vitro*, treatment of swine erysipelas with penicillin is unsuccessful. Good results, however, have been reported in turkeys—possibly due to the greater resistance of these birds to infection (see Gifford and Jungherr 1946, Woodbine 1950).

ERYSIPELOID

Infection of man with the bacillus of swine erysipelas was first described by Rosenbach in 1884 (see Rosenbach 1909). Though, according to Verge (1933), a cutaneous, an intestinal, and a generalized form of the disease may occur, the first type, usually referred to as "erysipeloid," is undoubtedly the commonest. It is met with in cooks, kitchen workers, butchers, and in those who handle fish, game, or cheese (Klauder 1926, 1932, 1938, King 1946). Epidemics of the disease have been reported. Stefansky and Grunfeld (1930) described an outbreak at Odessa affecting about 200 persons engaged in handling freshwater fish brought by sailing ships from the Dnieper and Bug, and Lawson and Stinnett (1933) in the United States reported 247 cases of erysipeloid occurring among workers employed in sawing and polishing bones in the manufacture of buttons.

Klauder (1932) says that infection can invariably be traced to contact with animals, fish, shell-fish, dead matter of plant or animal origin, or matter derived from animals such as hides, pelts, bones, and manure. The organism usually gains access through injuries of the skin, including the bites of animals, fish, and crustacea. Where the organism comes from, however, is not so clear. Direct transmission to man from swine suffering from erysipelas appears to be uncommon (Bierbaum and Gottron 1929), but it is sometimes met with in veterinary students (Morrill 1939). Circumstantial evidence suggests that the organism is present in the slimy coating of various fish, but, though its isolation from this source has been recorded by Schoop (1936), there is some doubt as to whether the slime is infected under natural conditions or whether it becomes contaminated from the flesh during preparation.

tests for the presence of antitoxin, the toxin is injected intradermally and, if not neutralized, gives rise to a local reaction. Such an antitoxic immunity is an immunity to the disease, but not to infection with, for example, diphtheria bacilli or streptococci, and the carrier state is quite as common in the immune as in the non-immune.

The presence of the bactericidal and lytic antibodies is clearly of considerable advantage to the host and these substances are also an obvious part of the defense mechanism. Similarly, the inhibition of multiplication of trypanosomes is an important part of the defense against certain species such as *Trypanosoma lewisi* (p. 769). Agglutinins and precipitins would appear on the surface to serve no useful purpose in the defenses of the immune animal, for example, bacteria are not killed by agglutination. The clumping of bacteria and the precipitation of soluble foreign protein, however, considerably facilitate the process of phagocytosis in that many bacteria may be engulfed at one time and insoluble masses of precipitated foreign protein may thus be removed.¹ In the case of pneumococcus antisera, for example, protective antibody as measured by mouse assay appears to be identical with precipitin and agglutinin. The pneumococcus antibody has been found to enter pneumonic lesions in rats, agglutinating the free pneumococci in the alveoli and thus stopping the spread of the infection.² Furthermore, heterologous protection may be demonstrated when portions of the antigenic mosaic are shared.³ Not all antibodies demonstrable by *in vitro* antigen-antibody reactions have protective qualities, however, in the case of the enteric bacilli protection appears to be associated with antibody to the heat-stable somatic antigens. It may be noted that in one instance there is evidence that precipitins play a direct part in defense; it has been found⁴ that the larvae of *Nippostrongylus muris* are stunted and immobilized in the skin of the immune animal with the formation of precipitates in and around them, and a similar reaction may be observed *in vitro*. In general, however, the precipitins and agglutinins function as a part of the defensive scheme in conjunction with the process of phagocytosis.

The importance of phagocytosis in resistance to infection is very great indeed and, in consequence, the opsonins assume particular importance from the theoretical point of view. In practice, though, opsonin titer is generally low as compared to antitoxin and other antibody titers and is highly variable; the value of the opsonic index as a measure of immunity is not great, possibly because of the use of polymorphonuclear leucocytes in *in vitro* tests. Opsonic activity, of course, involves the direct cooperation of certain cellular elements of the body, and any consideration of these antibodies, as well as agglutinins and precipitins, forces consideration of the cellular defenses and indicates the futility of any attempt to differentiate sharply between humoral and cellular immunity.

The implications of antitoxic immunity in diphtheria, tetanus and scarlet

¹ Cf. Cannon: *Physiol. Rev.*, 1940, 20:59.

² Wood: *Jour. Exp. Med.*, 1941, 73:201.

³ See the studies on the typhoid bacillus by Lippold: *Amer. Jour. Hyg.*, 1942, 36:354.

⁴ Saites and Tahaferro: *Jour. Inf. Dis.*, 1936, 59:207, Tahaferro and Saites: *Jour. Inf. Dis.*, 1939, 64:157.

sometimes contains small, round, pale-yellow or grey areas of focal necrosis, 1.5 mm. or less in diameter. The spleen is usually small, shrunken, pale pink, and rather tough; but in acute cases, and in cases in which there is extensive necrosis of the liver, the spleen is normal in size or slightly enlarged, soft, and of a dark purple colour. The adrenals are sometimes soft and diffuent. The mesenteric lymph nodes are always enlarged and œdematous. Culture of the organism is difficult; it may be successful from the mesenteric nodes; the heart's blood is usually sterile.

The disease can be reproduced experimentally, and is often characterized by a monocytosis, and focal necroses of the liver (p. 483).

DISEASES IN OTHER ANIMALS CAUSED BY ERYSIPELOTHRIX MONOCYTOGENES

Space does not permit a detailed description of the numerous types of disease to which this organism may give rise. Suffice it to say that it has been isolated by Pirie (1927) from a plague-like disease of gerbilles in South Africa, by Gill (1933, 1937) from an encephalitic disease of sheep in New Zealand known as "circling," by Jones and Little (1934) from cases of bovine encephalitis in New Jersey, by Tenbroeck (1932) from a sporadic disease of chickens in New Jersey, by Seastone (1935) in the United States and by Paterson (1937) in England from a generalized infection of fowls, by Biester and Schwarte (1939) from meningo-encephalitis of sheep in Iowa, by Biester and Schwarte (1940) from meningo-encephalitis of swine, by Paterson (1940) in England from abortion in sheep, and by Gifford and Jungherr (1947) from septicæmia in the wild raccoon in Connecticut (For further references see Paterson 1939.)

For cultivation of the organism from contaminated material, broth containing 1/2000 potassium tellurite is recommended, followed by plating after 6-24 hours at 37°C on to plain tryptose agar (Gray *et al.* 1950).

DISEASES IN MAN CAUSED BY ERYSIPELOTHRIX MONOCYTOGENES

Cases have been reported by Burn (1933-4, 1935, 1936) in the United States, by Gibson (1935) in Scotland, and by several other observers. Kaplan (1945), who reviewed the literature, collected records of 23 definite and 13 probable cases of human infection caused by this organism. Most of the true cases were characterized by meningo-encephalitis. In untreated cases the fatality rate was about 80 per cent. Post-mortem examination reveals a suppurative leptomeningitis and ependymitis, purulent otitis media, focal necrosis and patchy degeneration of the liver, swollen and congested spleen, focal necrosis of the adrenals, atelectasis, focal pneumonia and bronchiolitis. During life there is a mononuclear leucocytosis. The cerebrospinal fluid contains an increased number of leucocytes, mainly of the lymphocytic variety, and slender Gram-positive bacilli are found both inside and outside the cells. The disease may occur at different ages and has been observed in the new-born infant (Burn 1936). The mode of infection is unknown. In treatment sulphonamides appear to be of value, and aureomycin should be worth trying.

The relationship of *Ery monocytogenes* to glandular fever is discussed on page 2007.

fever noted above may be carried somewhat further. It is clear that the immunity so produced is what may be called an *effective immunity* in that it is an immunity against the infection or the disease produced by pathogenic bacteria. In addition to antitoxin, the antibodies to the various antigenic components of the bacterial cell may vary widely in their contribution to such an effective immunity. Thus, protection against pneumococcus infection is associated for the most part with antibody to the type-specific capsular antigen, while that to the somatic antigen contributes to only a relatively minor extent. On the other hand, in the case of enteric bacilli and the cholera vibrio effective immunity is produced by immunization with somatic or O antigen, and antibody to the flagellar antigenic complex has little protective action.

In certain instances the O antigen may be broken down somewhat further with respect to effective immunity. As indicated elsewhere (p. 452), the typhoid bacillus exists in two forms, the V form which contains Vi antigen, and the W form which does not. Vi-containing strains which lack typhoid bacillus O antigen can be obtained by dissociation, and certain other bacteria, notably a widely used strain of *Salmonella ballerup*, contain Vi antigen but are unrelated to the typhoid bacillus with regard to the specificity of the O antigen. Immunization with Vi-containing bacteria which lack the typhoid bacillus O antigen, whether typhoid bacilli or not, will protect against infection with V strains of the typhoid bacillus, but not with W strains. Conversely, immunization with a W strain of the typhoid bacillus will protect against infection with either V or W forms of this organism, but not against infection with Vi-containing bacteria that lack the O antigen of the infecting strain. It is concluded, therefore, that antibody to the O antigen and that to the Vi antigen are concerned in protection. The components of the other O antigenic complexes occurring in the *Salmonella* group (p. 433) are not sharply differentiated in that all seem to contribute to an effective immunity. The antigens so related to the effective immune response are sometimes spoken of as "essential immunizing antigens." When they are shared by related bacteria, such as *Salmonellas*, or phylogenetically unrelated microorganisms (p. 328), a cross immunity results, but when they are not shared by even closely related bacteria such as the various types of pneumococci, the cross immunity is of a low order. It follows, of course, that not only should an effective immunizing preparation contain the significant antigens, but antigens extraneous to the development of an effective immunity may be omitted. The first consideration has indicated the use of typhoid vaccine prepared from Vi-containing strains, and the second is the basis of the endotoxoid vaccines such as those prepared from the plague bacillus.

The extent of cross immunity also determines the polyvalency of vaccines, i.e., the number of bacterial strains which enter into its preparation. This is of obvious practical significance as in the preparation of dysentery vaccines. Such vaccines are usually quite toxic and if they must be made highly polyvalent, each additional strain or type of dysentery bacillus contributes its toxicity, with the result that the toxicity of the preparation increases if the amount and therefore antigenicity of the components are not reduced.

Cellular Immunity. The role of phagocytic cells in resistance to infec-

CHAPTER 59

TUBERCULOSIS

IN 1868 Villemin published his masterly study on the epidemiology of tuberculosis, and succeeded in transmitting the disease to animals by direct inoculation. Koch in 1882, after an investigation that will always remain as a classical example of thorough and accurate bacteriological technique, showed conclusively that the tubercle bacillus was the one essential cause of tuberculosis. The bacillus was resistant to all ordinary stains; but Koch succeeded in staining it by an alkaline solution of methylene blue, kept in contact with the tuberculous tissue for 24 hours. The bacillus would not grow on any ordinary medium; but he devised a new medium—inspissated blood serum—on which, after a delay of 10 days or so, growth first became apparent. Finally by a large series of inoculations with pure cultures of the bacillus, several generations removed from the primary one, he transmitted the disease to numerous animals of different species. Henceforward the demonstration of the bacillus afforded the sole infallible criterion for the diagnosis of all the manifold lesions of tuberculosis.

THE BACTERIOLOGY OF TUBERCULOSIS IN MAN

Tubercle bacilli may be divided by cultural and pathogenicity reactions into five types—human, bovine, murine, avian, and cold-blooded (see Chapter 16). Of these only the first two are found in natural infections of man. It is true that natural infections with the avian type have occasionally been described by continental workers, but the bacteriological evidence provided has not always been convincing. Feldman (1938), who reviewed the literature, could find a record of only 13 cases in which infection was probably due to the avian bacillus. In this country it was not found once in over 7,800 cases examined (Griffith 1938), but since then one case has come to light (Bradbury and Young 1946).

The extensive work of the Royal Commission on Tuberculosis (Report 1911a), supplemented by that of A S Griffith and other investigators, provided us with figures on the proportion of cases of tuberculosis in human beings caused by the bovine type of tubercle bacillus. This information was summarized in Table 92 of the third edition (1946) of this book. More recent figures obtained by special surveys carried out in England and Wales during the second world war yielded results that were substantially in accordance with, though rather lower than, the previous ones (Report 1949), they are reproduced in Table 91. It will be seen that in non-pulmonary tuberculosis the majority of infections due to the bovine type of bacillus occur during the first fifteen years of life, and that, taking all ages into consideration, about 1·4 per cent. of pulmonary and 25 per cent. of non-pulmonary infections are caused by this type of organism. The gastro-intestinal

tion was established by Metchnikoff⁵ on the basis of studies on lower forms such as the water flea, *Daphnia*. He distinguished two types of cells, the *microphages* or polymorphonuclear leucocytes (heterophils) and the *macrophages* or large mononuclear cells occurring both free in the blood stream and fixed as tissue cells. The former were regarded as of prime importance in the bacterial infections, while the macrophages were thought to be only indirectly active except in chronic infections such as tuberculosis and leprosy. With advances in knowledge, however, it has become clear that although their immediate mobilization accentuates the limited significance of the polymorphonuclear leucocytes, the variety of fixed and free connective tissue cells of mesenchymal origin, grouped under the general head of macrophage, is of considerably greater importance in resistance to disease.

The distribution and interrelationships of these phagocytic cells may be considered briefly in outline form:⁶

A. The predominantly fixed cells of the reticular and loose connective tissue which can be divided into two great groups

- (1) fixed and free macrophages, including the reticular cells, and
- (2) the fibroblasts of connective tissue and the endothelial cells lining the ordinary blood vessels

- (1) Macrophage is essentially a physiological designation for almost any large mononuclear connective tissue cell which is predominantly phagocytic and includes

(a) fixed cells such as

1. *pericytes* (Maximov), fixed, undifferentiated outstretched cells in the adventitia of all the small blood vessels of loose connective tissue throughout the body.
2. *reticular cells* which, with fibers, form the stroma of all reticular (myeloid and lymphatic) tissues, and
3. *littoral cells* (Siegmund) which line the sinuses of the reticular tissues, liver, hypophysis and adrenal. In the liver these are designated as *Kupffer cells*. Although frequently called endothelial cells or cells of the special endothelium, these are in fact either true reticular cells or have greater developmental potencies than ordinary endothelial cells.

These cells can divide by mitosis, become phagocytic, and develop into fibroblasts or practically any other blood or connective tissue cell. Here it is important that they can become phagocytic either in their fixed position (fixed macrophages) or after rounding up and becoming free (free macrophages).

- (b) The free cells occurring in the loose connective tissue are variously known as *histiocytes*, *clasmatoocytes*, *rhagiocrine cells* or wandering resting cells, are either phagocytic or can become so without morphological change, can reproduce by mitosis, and transform into fibroblasts.

- (2) The fibroblasts and endothelial cells are morphologically characterized by outstretched, ill-defined cytoplasm, a large oval vesicular nucleus containing dust like chromatin granules, and small nucleoli

(a) The fibroblasts divide by mitosis but do not develop into other cells (except in bone and cartilage) and are rarely phagocytic although instrumental in repair and walling off foreign material.

(b) The endothelial cells which line the larger blood vessels and capillaries (not including the littoral cells) likewise are rarely phagocytic but may be transformed into fibroblasts.

⁵ Metchnikoff, *Immunity*, Cambridge, 1905.

⁶ This material is taken, with only minor modifications, from Tahaferro *The Immunity of the Parasitic Protozoa*, Chapter XVIII in Calkin's *Protozoa and Biological Research*, Columbia University Press, New York, 1911.

been shown that in Scotland and in Northern England an appreciable proportion of cases are due to infection with the bovine type (Table 92). The reason for the differences between different parts of the country is to be found in the higher incidence of tuberculosis in cattle in the northern half of Great Britain.

TABLE 92

FREQUENCY OF BOVINE TUBERCLE BACILLI IN PULMONARY TUBERCULOSIS IN DIFFERENT PARTS OF GREAT BRITAIN AND IRELAND.

(Cumming 1935, Griffith and Smith 1940, Griffith 1941, Griffith and Munro 1944, Cutbell and Lynn 1944.)

Area	No. of Strains Examined.	Percentage Bovine
Scotland, North-east	972	7.0
Rest of Scotland	1,797	5.1
England, North and Middle	3,219	2.0
Wales	201	1.0
England, South	989	0.6
Ireland	320	0.0

EPIDEMIOLOGY

Tuberculosis is an infectious disease that spares neither age, sex, race, nor nationality. The pulmonary form was described by Hippocrates, and spinal caries has been observed in the mummies of ancient Egypt (Smith, E, 1909). With the spread of civilization it became more rife; but now, in the more highly civilized countries of the world, it is declining.

Mortality from Tuberculosis.—Table 93 sets out the prevalence of tuberculosis in different countries at the beginning of the second world war. The crude death

TABLE 93

CRUDE DEATH RATES PER 100,000 POPULATION FROM ALL FORMS OF TUBERCULOSIS IN 1939 (Modified from Yelton 1946.)

Prevalence	Country	Death Rate	Prevalence	Country	Death Rate
Very low under 50 per 100,000	Denmark	34	Medium: 100-149 per 100,000	Austria	100
	Australia ¹	40		Eire	113
	Netherlands	41		Czechoslovakia	124
	United States	47		France	137
				Hungary	148
Low: under 100 per 100,000	Germany	50	High: 150 and over per 100,000	Russia	160
	Canada	53		Romania	162
	New Zealand ¹	60		Finland	190
	England and Wales	62		Poland	195
	Belgium	68		Newfoundland and Labrador	198
	Scotland	70		Japan	207
	Sweden	75		Brazil	250
	Switzerland	80		China	400-500
	Northern Ireland	84		Greenland	550
	Norway	86			

¹ White population only.

Note.—Some of the figures in the high prevalence group are only approximate

B. The free connective tissue and blood cells.

- (1) The cells of the blood and lymph are classified as to whether of myeloid or lymphoid origin:
 - (a) The *lymphoid cells* of the blood include the various sized lymphocytes which together with monocytes, are termed *agranulocytes*. They divide mitotically and can develop into macrophages with all the latter's developmental potencies. As they become transformed into macrophages, they show increased amounts of cytoplasm, their nuclei take on macrophage characteristics and they become phagocytic. These transitional forms are known as *polyblasts*.
 - (b) The *myeloid cells* are the various *granulocytes* (heterophils or polymorphonuclears, eosinophils and basophils), the erythrocytes and platelets. Of these the heterophils are functional in immunity by virtue of their phagocytic activity but are "end" cells which do not reproduce or develop into other cells.
- (2) The free mesenchymal cells are lymphoid cells indistinguishable from lymphocytes which occur in varying numbers in reticular and loose connective tissue and here act as precursors or "stem" cells of lymphoid and myeloid cells and hence may give rise to macrophages. They are variously termed *lymphocytes*, *hemocytoblasts*, *lymphoblasts*, *myeloblasts* or *monoblasts* according to varying theories of blood formation. Under normal conditions lymphocytes in lymphatic tissue give rise only to lymphocytes and hemocytoblasts in bone marrow only to myeloid cells but under abnormal stimuli they may exhibit their full potencies for development. These stem cells are self-perpetuating, but as noted above, may arise from the fixed mesenchymal cells.

The Systems of Cells. It will be apparent that the cells primarily associated with defense against invading microorganisms are widely distributed through the body in the blood and lymph, cartilage, bone, reticular (blood forming) tissue of the myeloid and lymphatic organs and in the loose connective tissue associated with the skin, omentum, liver, lung, etc. It is customary to group the macrophages into so-called systems of cells, the best known of which is Aschoff's⁷ *reticulo-endothelial system*. The term is not a good one, however, for, as indicated above, the cells of the endothelium proper are not phagocytic, although called endothelial cells, the phagocytic littoral cells (such as Kupffer cells) are similar to or identical with reticular cells. Perhaps a better designation is simply *macrophage system* or the broader term *lymphoid-macrophage system*,⁸ which includes the lymphoid cells and the transitional polyblasts.

Local Defense. The cellular response to an inflammatory stimulus⁹ is characterized by an initial migration of polymorphonuclear leucocytes to the point of injury. These cells are not numerous and soon disappear when the inflammatory material is sterile, but when bacteria are present they continue to migrate from the blood vessels and actively phagocytose the invading microorganisms. The functions of this first line of defense, although important, are strictly limited, since the cells are short-lived and do not multiply *in situ* but must be continuously recruited from the blood stream.

More important to local defense are the lymphoid cells of the blood and lymph, the lymphocytes and monocytes, which also migrate from the blood vessels but which, unlike the polymorphonuclear leucocytes, are long-lived

⁷ A. A. Aschoff, *Ann. N. Y. Acad. Sci.*, 1934, 26-1.

⁸ Research Memoir No. 29, 1937.

The Macmillan Company, New York.

Wright (1939) likewise stress the importance of poverty, poor housing conditions, and overcrowding. That biological factors are also operating, however, is almost certain. Ostenfeld, Heitmann, and Neander (1931), for example, point out that in Sweden during the past century there has been a centrifugal spread in the frequency of the disease. A hundred years ago the highest death rate from tuberculosis was in Södermanland, where now it is lowest, whereas Norbotten, which 100 years ago was relatively free from the disease, is now specially affected. The suggestion is that the resistance of a population in which the disease has been prevalent for some generations is greater than that of a population coming into contact with tuberculosis for the first time. Further evidence in support of this thesis will be found in the section dealing with tuberculosis in primitive races.

Wolff (1930b) lays great weight on the importance of diet. Tuberculosis, almost alone among the infectious diseases, seems to be profoundly influenced by malnutrition and starvation, though there is little exact knowledge of the mechanism by which this effect is produced. Shortage of first-class proteins was regarded by Faber (1938) as being largely responsible for the rise in tuberculosis mortality that occurred in western Europe during the war of 1914-18. Experimentally there is some evidence that animals fed on a plentiful diet are more resistant to infection than those on a meagre diet, and that Vitamin A deficiency may lower their resistance (see Robertson 1934, Clausen 1934, and Chapter 54). Kon and Maddock (1938), however, were unable to confirm the importance of Vitamin A in the protection of pigs against experimental infection. Osborn and Gear (1940) have pointed out that animals, like the dog and the rat, which can synthesize their own Vitamin C, are more resistant than those, like man, the monkey, and the guinea-pig, which cannot. During the war of 1914-18 the death rate from tuberculosis in German cities, which was only 1.57 per 1,000 in 1913, rose in 1918 to 2.87. In Warsaw by 1917 it had reached 8.4 per 1,000, and in Belgrade, as the result of the military occupation and want of food.

In the second

have played a part in this rise in Great Britain are discussed in a report by the Imperial Research Council (Report 1942).

Without in any way minimizing the desirability of active public measures against tuberculosis, it may be pointed out that the decline in the tuberculosis death rate in this country set in long before the establishment of hospitals, sanatoria, or dispensaries for the treatment of patients suffering from the disease, indicating that the underlying social, economic, and biological causes were able by themselves to effect a considerable reduction in the mortality from tuberculosis in the absence of any *ad hoc* public health interference.

Mortality from the pulmonary and non-pulmonary forms of tuberculosis varies greatly in different countries. In 1951 in England and Wales, 12,000 persons died from pulmonary and 1,775 from non-pulmonary tuberculosis. The non-pulmonary deaths, therefore, constituted about 13 per cent of the total. Approximately half the non-pulmonary deaths occurred under 15 years of age. In the United States of America non-pulmonary tuberculosis is relatively less common, accounting for only 8 per cent of the total deaths (Halpern 1918).

Other Factors affecting the Mortality from Tuberculosis.

Occupation.—Occupation is of importance. In this country the mortality from pulmonary tuberculosis is below the average for all males in fishermen and coal-miners, but is high in stone and slate quarrymen, in cutlers, file-makers, earthenware manufacturers, and highest of all in the Cornish tin-miners. All trades, in fact, in which the men are exposed to the inhalation of particles of metallic or stone dust, particularly dust containing silica, have a high mortality rate from pulmonary

and multiply in the tissues. These stem cells may be transformed to macrophages which, together with macrophages already present in the area, actively phagocytose and digest the invading microorganisms. When large bodies of foreign material are present, the macrophages may fuse to form foreign body giant cells, when microorganisms are indigestible, they may form giant cells around them such as the epithelioid cells of the tubercle. Their progressive development into fibroblasts supplies the active elements for regeneration and repair, the formation of scar tissue and the walling off of foreign bodies.

Certain other cells may take part in the late stages of the inflammatory process, the eosinophils, for example, may play a part in the detoxification of proteins and their disintegration products.

General Defense. When a stimulant is distributed over a large part of the body the reaction is regarded as a general rather than local one; such distribution, however, usually means that the stimulant is in the blood stream and is combated by the cells of organs most closely associated with the blood, i.e., the spleen, liver and bone marrow, and in some cases general reactions may be regarded as local ones in strategically placed organs. The same types of cells are involved as in the local reaction, the polymorphonuclear leucocytes being mobilized first, with the macrophages playing the more important role. Endothelial cells, although in contact with blood-borne material, show very little phagocytic activity and the fibroblasts are rarely active.

The Cellular Response in Immunity. The above reactions, which are observed to occur in the non immune animal, are markedly accentuated in the immune animal, and there is an increase in the number of macrophages frequently designated as a hyperplasia of the reticulo-endothelial system. The cooperative role of the humoral antibodies is of considerable significance; an antigen is localized by agglutination if cellular or by precipitation if in solution, and in either case the material is made more readily phagocytatable by opsonization. Bacteria injected into the blood stream, for example, are rapidly removed in the immune animal by the fixed macrophages of the liver and spleen, and staphylococci injected into the skin are localized and phagocytosed in great numbers.¹⁰ When antigen and antibody meet in the tissues of an immune animal, there are not only localization and opsonization of the antigen, but also a much heightened inflammatory reaction including a speeding up of the cellular response.

The Site of Formation of Antibodies. The question of the site of antibody formation is an old one and, since it now appears to be definitely established that antibody is modified serum globulin, it becomes one of the loci of globulin synthesis. Evidence has accumulated in support of the theory that antibody is formed in cells rather than from the humoral constituents of the blood, and that the cells responsible for the synthesis of immune globulin are those of the macrophage system, especially in the liver, spleen, lymph nodes and bone marrow. This evidence, generally indirect, is of a number of kinds. Thus, antibody formation is seriously interfered with by the removal of tissues containing large numbers of macrophages such as

¹⁰ Cf. Cannon *Physiol. Rev.*, 1940, 20 89

century and the first decade of the present century the mean annual death rate per 100,000 living was highest in infancy and early childhood, whereas in 1951 it was highest in the 55-65 age group.

The fall in the death rate during the early years of life has been most striking. This is almost certainly attributable to some extent to the much greater care now exercised in infant welfare, and to the more general provision of heat-treated milk (see Drolet 1930). It may be noted that the highest tuberculosis mortality in children occurs during the 2nd year of life, and is due to various types—meningeal,

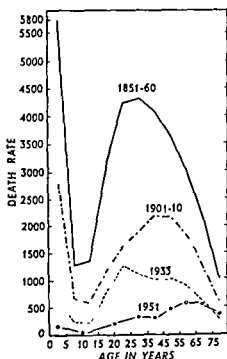


FIG. 274.

Death rates per million from tuberculosis (all forms) at ages 0 to 75 in England and Wales.

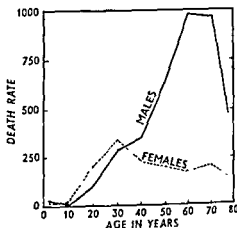


FIG. 275.

Respiratory tuberculosis in England and Wales. Death rates per million by age and sex in 1951.

abdominal, miliary, respiratory and disseminated. Meningitis alone is responsible for about 40 per cent. of the total deaths during the first ten years of life.

The death rate in young adults, on the other hand, has undergone much less alteration. Most of the deaths in this group are due to pulmonary tuberculosis, and, as Fig. 275 shows, the death rate from this form is rather higher in females than in males in the 15-35 age group. After the age of 35 the male pulmonary tuberculosis death rate increases, till in the 55-65 age group it is over five times that of the female death rate. The difference between the male and female mortality rates has been increasing steadily for many years. In 1851-60 the age period of maximal tuberculosis mortality in males was actually lower than in females—20 to 25 as against 25 to 35. It has gradually risen in males to 55 to 65, and fallen in females to 20 to 25. These changes are most puzzling and are the subject of considerable speculation (Stocks 1949, Springett 1952, McDonald 1952). They are not confined to this country, but are reflected in greater or less degree in the statistics of many other European countries and of the United States of America (see Dow and Lloyd 1930, Drolet 1930, Whitney 1931, MacNalty 1932, Hill 1936, Hart and Wright 1939, Pitney and Kasius 1947, Halpin and Turner 1951).

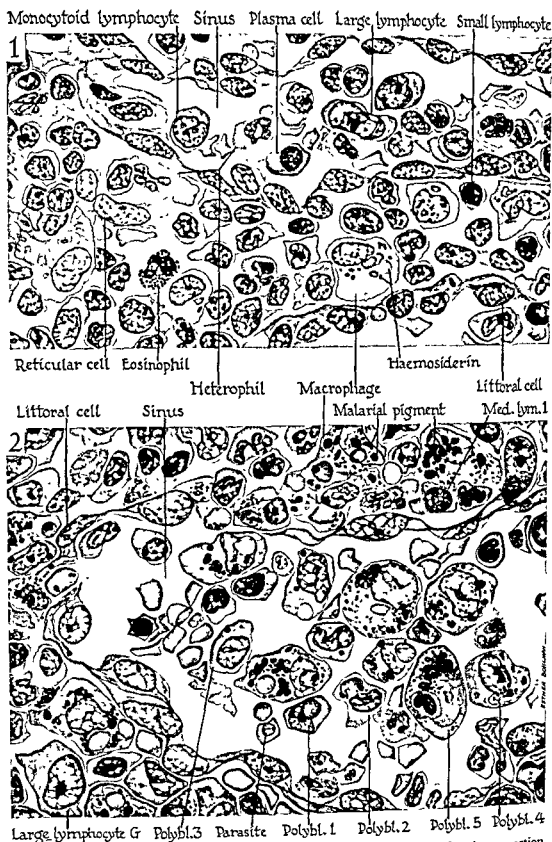


Fig. 39. Cells of the macrophage system. Sections of monkey spleen showing a portion of a venous sinus and a Billroth cord in the red pulp. 1, Normal monkey; note the typical structure of the non-granular leucocytes, the reticular cells with indeterminate cytoplasm, and the rounded macrophages within the cord. 2, Monkey killed ten days after infection with *Plasmodium cynomolgi*, note the large phagocytic cells absent from the normal spleen, the series of polyblasts labelled Polybl. 1-5 illustrate the progressive hypertrophy of the non granular leucocytes into macrophages. Hematoxylin and eosinazure II, $\times 1240$ (Taliaferro and Mulligan).

in which the morbidity from tuberculosis among nurses living and working in the same hospitals was two and a half times as high among those of Irish and Welsh as among those of English origin. The observations on inherited resistance, described in the next section, leave little doubt that there is a genuine difference in the susceptibility of different races to tuberculosis, depending to some extent on the length of time they have been in contact with the disease.

It may be pointed out that primitive peoples who have had little experience of the disease often suffer severely when they leave their native country and take up residence in a civilized area (Cummins 1908, 1911-12, Westernhoeffner 1911, Borrel 1920, Bushnell 1920). Not only is the case incidence and mortality high, but the disease tends to pursue a rapid course accompanied by enlargement and caseation of the lymphatic nodes and early generalization resembling far more the acute type of disease met with in infancy than the localized, slowly spreading, ulcerative type of phthisis common in adults of civilized races.

There must be few native races left that have not had some degree of contact with civilization; and it is to be expected that, as this increase, the acute military type of tuberculosis will give way, in course of time and as the result of natural selection, to the more chronic types characteristic of races that have been long in contact with the disease. (For references to tuberculin surveys in primitive peoples, see numerous papers in the *American Review of Tuberculosis* and the *Tropical Diseases Bulletin* from about 1928 onwards.)

Heredity.—That tuberculosis often runs in families is a well attested observation. How much of this can be explained by inferior genetic resistance, how much by increased household exposure to infection, and how much by chance alone, is difficult to decide. Influenced partly by the behaviour of the disease in primitive peoples encountering tuberculosis for the first time, and partly by the careful investigations of Carmichael and of Lurie on animals and of Kallmann and Reisner on human beings, the tendency of late years has been to pay considerably more attention to the influence of heredity than at any time since the discovery of the tubercle bacillus.

Carmichael (1938), for example, found that the incidence of tuberculosis among the long-horned Hamitic cattle belonging to the Bahima people of Ankole was much higher than among the humped Zebu cattle of Uganda, even though the environmental conditions appeared to favour the Hamitic variety. Though these animals lived almost entirely in the open air, 80 per cent. of them reacted to tuberculin, as against less than 1 per cent. among the Zebu cattle. Besides being resistant to natural infection, the Zebu cattle proved almost entirely refractory to experimental inoculation. Lurie (1941) studied six inbred families of rabbits, and found a wide difference in their response to infection with bovine tubercle bacilli, whether inhaled as the result of natural contact or inoculated parenterally. In the animals of the most resistant family the lung disease tended to be localized, and the primary focus progressed slowly, became encapsulated, and underwent cavitation. In the animals of the most susceptible family little or no localization occurred, and the disease was of the rapidly progressive generalized type. It is important to point out that in all the animals the disease was progressive; the chief difference lay in the rate of progression. It was also found that, when the bacilli were administered artificially by the respiratory tract, the difference between the families was noticeable only when the dose was small; above a critical level the rabbits of even the most resistant family succumbed rapidly to the infection. No one factor seemed to be responsible for the differing resistance of the different rabbit families. Several factors were apparently implicated. Thus it was found that low skin permeability to the injection

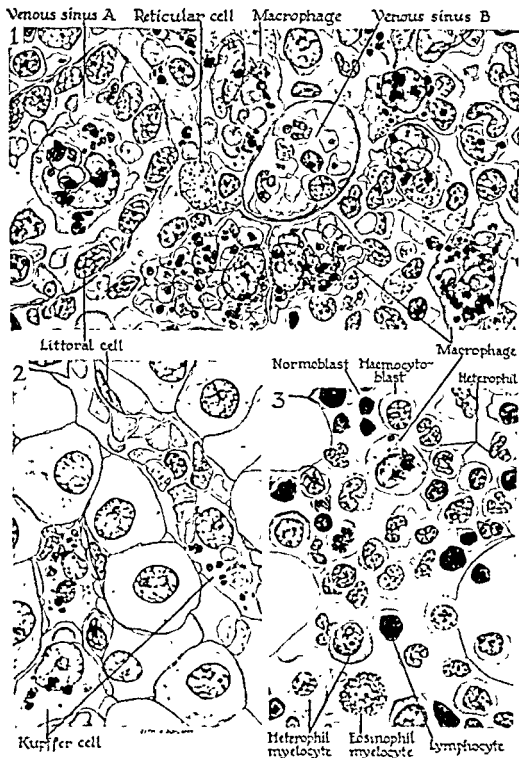


Fig. 40. Cells of the macrophage system. Sections of spleen, liver and bone marrow from a monkey killed fifteen days after infection with *Plasmodium cynomolgi*. 1, A Billroth cord and two venous sinuses from the spleen; note the phagocytic activity of the macrophages and smaller polychroms. 2, Section from the liver; the Kupfer cells are phagocytic but here less active than the spleen macrophages. 3, Section of bone marrow, showing a single, relatively small macrophage. Hematoxylin and eosin azur II $\times 1240$ (Tahaffarz and Mulligan).

primary infection by the tubercle bacillus, the genetic constitution of the individual plays by far the greatest part in determining whether he develops clinical tuberculosis or not—assuming, of course, that for the purposes of comparison the general environmental conditions can be considered approximately constant.

The Prevalence of Tuberculosis.

Though tuberculosis is a notifiable disease in this country, large numbers of cases are not in fact notified, and the Registrar-General has, since 1931, ceased to publish the returns in his annual report. To obtain a satisfactory idea of the prevalence of tuberculous infection, resort must be made to three other sources of information—the tuberculin test, post-mortem statistics, and X-ray examination.

Latent Tuberculosis as revealed by the Tuberculin Test.—Von Pirquet (1907) in Vienna drew attention to the value of the tuberculin test in indicating the distribution of infection through-

a given population. Von Pirquet's skin test consists in placing a drop of tuberculin on the forearm and a drop of 50 per cent. glycerine adjacent to it as a control. With a small lancet a scratch is made through each of the drops. A positive reaction is denoted by the appearance within 24 or 48 hours of a bright red papule, at least 5 mm in diameter, at the site of inoculation; later the colour becomes dark red, and the lesion disappears in about a week. This is known as a primary positive reaction. In some persons the reaction is delayed for a few days, or occurs only after a second inoculation a week later—torpid or secondary reaction.

A primary positive reaction is taken as evidence of the existence in the body of a comparatively recent tuberculous lesion, which may or may not be actively progressing. A torpid or secondary reaction is believed to indicate the presence of an old tuberculous lesion, inactive or in process of

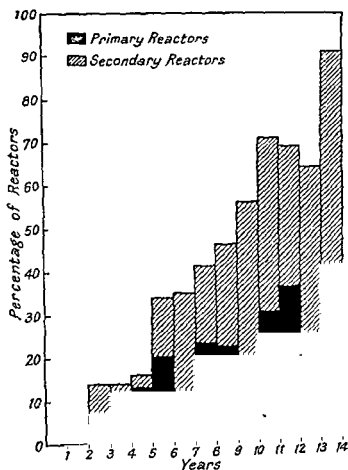


FIG. 277.

(Modified from von Pirquet.)

healing. Complete failure to react is evidence that the subject has never been in contact with tuberculosis, or that healing has occurred.

Using this test, von Pirquet (1909) examined 1,134 clinically non-tuberculous infants and children belonging mainly to the poorer classes at a time when tuberculosis was rife in Vienna. The results are summarized in Table 96, and represented graphically in Fig. 277.

The figures for the older age groups are rather small; but it is clear that by

the spleen. Similarly, blockade of the macrophage system by the injection of india ink (colloidal carbon) or other substances which are phagocytosed to such an extent that the phagocytic cells are fully occupied inhibits the immune response. Likewise, injury to hematopoietic tissue by irradiation, inoculation of benzene or nitrogen mustards also decreases the antibody response.

If it be true that these cells are responsible for the synthesis of antibody, it would appear to follow that they would form immune globulin in tissue culture. Carrel and Ingebrigtsen¹¹ found that the addition of goat red cells to cultures of guinea pig bone marrow and lymphoid tissue resulted in the appearance of hemolysin which, however, did not require complement for lysis. It has not been possible to repeat this work, i.e., the addition of antigen to tissue culture of cells from normal animals, but many workers have observed the formation of antibody in cultures of tissue fragments, such as spleen and bone marrow, taken from animals that had been previously inoculated with antigen.

The inoculation of antigenic substances results in a marked lymphoid proliferation in the regional lymph nodes and in the spleen. In recent years observations have been reported by two groups of workers, Dougherty and his associates¹² and Ehrlich and his associates,¹³ which suggests a function of the lymphocyte in either the storage or formation of antibody. The evidence is of the following general nature¹⁴: Typhoid bacilli or sheep erythrocytes were injected into the plantar surface of the hind feet of rabbits; lymph was collected from the efferent lymph vessel of the popliteal lymph node, and the lymphocytes separated and washed by centrifugation; saline extracts were prepared by alternate freezing and thawing of the suspension of washed lymphocytes. Comparison of the antibody titer in the lymph plasma and lymphocyte extract showed that, five days after injection, the extract contained 8 to 16 times as much antibody as the plasma. In view of the long persistence of a solid immunity, this concept is surprising in that it relates antibody formation to a tissue as structurally and functionally labile as lymphatic tissue. This area is being actively investigated by a number of workers and in time no doubt the role and relative importance of the lymphocyte will become clear.

Local Immunity. The defense mechanisms functioning in the immune state have been assumed to be general ones and, for all practical purposes, equally effective throughout the body. It has been suggested, however, that these mechanisms are localized, or obviously accentuated, in certain tissues, not necessarily in tissues containing a large proportion of cells of the macrophage system, but tissues such as the skin, the intestinal mucosa, the nasal mucosa, etc.

¹¹ Carrel and Ingebrigtsen. *Jour. Exp. Med.*, 1912, 15:287.

¹² Dougherty et al. *Jour. Exp. Med.*, 1944, 57:295, *ibid.*, 1945, 58:135, *ibid.*, review in *Ann. New York Acad. Sci.*, 1946,

¹³ Ehrlich et al. *Science*, 1945, 101:28; *Jour. Exp. Med.*, 1945, 81:73; *ibid.*, 1946, 83:373; *ibid.*, 1946, 84:157.

¹⁴ Experiments reported by Harris, Grimm, Mertens and Ehrlich. *Jour. Exp. Med.*, 1945, 81:73.

of dosage of tuberculin is overcome by expressing the potency of preparations of tuberculin in terms of the International Standard Old Tuberculin and of the International Standard Purified Protein Derivative (Mammalian) (see p. 1519). The potency of the standards in both cases is expressed in international units. The international standard of old tuberculin, which is an average preparation of the substance, contains 100,000 units/ml. This value was adopted for convenience, so that the smallest dose in the routine Mantoux test, 0.1 ml. of a 1/10,000 dilution, contains approximately 1 unit.

Numerous surveys have been carried out in different countries during the past twenty years or so by the Mantoux test. The results of three of these are represented in Fig. 278. Observations on probationer nurses entering hospitals during

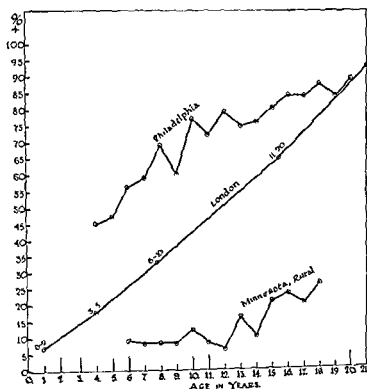


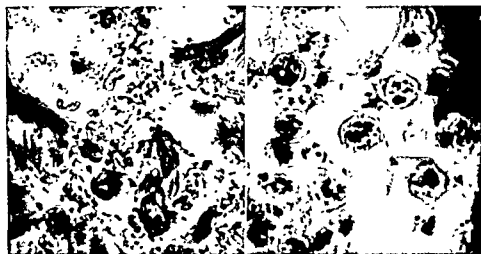
FIG. 278.

to von Pirquet test (Slater and Jordan 1935).

the 1930's showed that about 80-90 per cent. in London, Sweden, and Finland, 60-70 per cent. in Denmark, and 10-60 per cent. in the United States were tuberculin-positive (see Report 1918a). Similar figures have been reported for students entering university, except in the United States where the positive reactors numbered only 30-50 per cent. (see Long 1938). More recent observations from the United States have revealed a tuberculin-positive rate among nurses ranging from only 8 per cent. in Minneapolis to 23 per cent. in Philadelphia, showing a big decline in the infection rate (Goddard, Edwards and Palmer 1949).

In the large industrial cities of Europe, and to a less extent of America, most children are probably infected with tuberculosis by the time they reach 18 years of age, but in rural areas infection is much less frequent. Since only a comparatively small proportion of reacting children develop clinical tuberculosis or die of

Although attributable in some degree to the nature of the local defense factors, such as pH and the like, the predilection of an invading micro-organism for some particular part of the body such as the central nervous system might be regarded as indicative of a relative susceptibility of certain tissues and, conversely, a relative resistance on the part of others—in Ehrlich's terminology, the presence or absence of cell receptors. In a disease such as typhoid fever or erysipelas, for example, it should be necessary then that only the intestinal mucosa or the skin be immune, the other tissues being already resistant. This concept has been advocated particularly by Besredka.¹⁵ The evidence upon which it has been based is generally regarded



as relatively weak, and local immunity in Besredka's sense probably does not exist.

There is, however, good reason to think that local immunity, not of a particular tissue no matter where it may be in the body such as the skin, but of a local area, does exist. It would appear to follow that the presence of antigenic material in a particular area would stimulate the production of antibody by the cells of that area and, in consequence, higher antibody titers would be found in the inoculated and immediately adjacent area than in the blood stream and other tissues. Such higher antibody titers have been demonstrated¹⁶ in the nasal mucosa, for example, and it appears probable that, under circumstances in which antibody production takes place within a restricted area, that area may exhibit an accentuated immunity.

Natural Immunity. As pointed out elsewhere (p. 216), the defenses of the normal animal against infection are of two general types. The first,

¹⁵ Besredka: *Local Immunization*, Williams & Wilkins Company, Baltimore, 1927.

¹⁶ Cann *et al.*: *Proc. Soc. Exp. Biol. Med.*, 1931, 29: 517, 675; *Hartley: Jour. Inf. Dis.*, 1940, 66: 44.

73.3 per cent. had died of the disease (Table 97). In other words, latent or non-lethal tuberculosis is very much commoner in adults than in early life.

A similar, but more extensive, series of autopsies on a working-class population was reported by Burkhardt in Dresden in 1906. The results agreed fairly well with those of Naegeli, the chief differences being that tuberculosis at Dresden was commoner but less fatal in children and more fatal in adults than at Zurich.

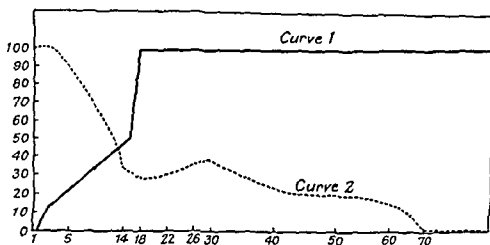


FIG. 280.

Curve 1 = frequency of tuberculous lesions in every 100 autopsies of the same age group.
Curve 2 = frequency of fatal tuberculosis in relation to all cases of tuberculosis.

(After Naegeli.)

Burkhardt gives a very instructive table recording the incidence of latent tuberculosis in the different age groups (Table 98).

From this table it is seen that, though the percentage of persons with latent active tuberculosis does not vary greatly in the different age groups, the percentage with latent inactive or healed tuberculosis increases progressively with age.

TABLE 97

MODIFIED FROM NAEGELI (1900), SHOWING THE FREQUENCY OF TUBERCULOSIS (Tb.) OF ALL FORMS IN POST-MORTEM SUBJECTS AT ZURICH.

	Autop- sies.	Tb not found	Tb. found.	Per- centage Tb.	Of Tb. Patients			
					Died of Tb.	Per- centage.	Non- lethal Tb	Per- centage.
Children 0-18 years	88	73	15	17.04	11	73.3	4	26.7
Adults 18 years and over	420	29	391	93.11	110	28.13	281	71.84

The general inferences that appear to be justified by these results are as follows:

- (1) Tuberculosis is uncommon, but often fatal, in infancy.
- (2) The incidence of tuberculous infection increases steadily in childhood, but the disease appears to be least fatal during this period.
- (3) After school-leaving age the incidence rises rapidly, reaching 90 per cent. or so by the age of 18 years. The case-fatality rate also increases.

characterized by non-specificity with respect to the infectious agent, has been called resistance and discussed earlier. The second type, showing a greater or lesser degree of specificity, is considered here as natural immunity.

It is the rule rather than the exception that pathogenic microorganisms are sufficiently closely adapted parasites that they are able to establish infection only in more or less closely related species of hosts. Thus it is perhaps not to be expected that the same parasite will be able to infect organisms as widely different as higher plants and mammals. It is of particular interest, therefore, that *Pseudomonas pyocyanea*, a not uncommon pathogen of man and higher animals, has been found able to infect tobacco plants and may be identical with the plant pathogen *Phytophthora poly-*
color.¹⁷

While in Ehrlich's terminology it might be said that the cellular organization of the plant lacks receptors for the human pathogen, there is evidence of a positive type of resistance to such infection. Antibiotic substances, similar to those produced by bacteria and fungi (p. 128), have been found in a number of higher plants. Thus, extracts of cabbage, turnip, onion and barberry plants inhibit the growth of *Bacterium coli* and *Bacillus subtilis*,¹⁸ chlorophyll and related compounds have been found to inhibit the growth of the tubercle bacillus,¹⁹ and substances which inhibit the growth of *Staphylococcus aureus* and *Bacterium coli* have been found in a large number of angiosperms.²⁰ None of the plants, however, is known to produce anything resembling antibodies.

Natural immunity is closely associated with the specific immune response, an immunity which is expressed in the form of the bactericidal and opsonic powers of normal blood, in the presence of the so-called normal agglutinins and the like. Such antibodies are generally present only in low titer, and the immunity associated with their presence in man is of a low grade but is possibly a significant part of that complex designated "resistance" in an earlier chapter. In many instances antibodies may be found under circumstances in which it is highly improbable that the animal has come in contact with the infectious agent; it has been noted, for example, that cattle sera will not infrequently neutralize the yellow fever virus and, more often, contain agglutinins for bacteria such as the typhoid and dysentery bacilli, the cholera vibrio, and certain of the rare varieties of *Salmonella*. Although present in low titer, such normal agglutinins appear to be no different from the immune agglutinins in adsorbability, heat resistance and the like. The normal macrophage response does not involve this specific element, for all kinds of intact particles are phagocytosed. The question of the origin of these antibodies is, then, of some significance and it may be asked whether or not specific antibodies may be formed in the absence of antigenic stimulation.

¹⁷ Elrod and Braun: Jour. Bact., 1942, 44:633.

¹⁸ Cf. Sherman and Hodge: Jour. Bact., 1936, 31:96; Dick: Arch. Surg., 1940, 41:287; Fuller and Higgins: Food Research, 1940, 5:503.

¹⁹ Daly, Heller and Schneider: Proc. Soc. Exp. Biol. Med., 1939, 42:74.

²⁰ Osborn: Brit. Jour. Exp. Path., 1943, 24:227; see also Pratt et al., Science, 1944, 99:351; Tsuchiya et al.: Jour. Bact., 1944, 47:422; Lucas and Lewis: Science, 1944, 100:597.

tuberculin reactors in the first two years of life was nearly the same as the percentage incidence of tuberculous lesions found at autopsy, indicating again that at this age practically all children who became infected died of the disease. These figures refer to a low social class in Scotland in which tuberculosis is undoubtedly a very severe disease, and it would be unjustifiable to conclude that they are valid for all classes and for all countries. Indeed there is considerable evidence to the contrary (Rosenberg and Kereszturi 1937, Price 1938, Miller 1947). With the progressive decline in tuberculosis mortality that is occurring in Europe and America, primary lesions are being increasingly observed in adults. Many of them appear to run a more benign course than those in infancy (Terplan 1934, Wallgren 1938).

In considering the *fatality of tuberculous infection in early life*, it must be remembered that the type of case seen by Naegeli, by Burkhardt and by Blacklock, was not representative of the whole infantile population, but merely of that portion of the infantile population which showed symptoms of disease of sufficient severity to demand admission to hospital. If an equal number of apparently healthy infants could have been examined, it is quite conceivable that many would have shown latent tuberculous lesions. All our figures hitherto have been selective. The diagnosis of tuberculosis in infancy is extremely difficult, and only advanced and fatal cases are recorded. The result is that we have come to believe that infection in infancy is usually fatal. That this conclusion is wrong is shown very clearly by the observations made at Lübeck on the infants who were vaccinated by the mouth during the first few days of life with the B.C.G. strain (see p. 1529). The vaccine had by mischance become contaminated with virulent human tubercle bacilli and a large number of the infants became infected (Report 1935b). Out of 251 vaccinated infants, 72 died of tuberculosis, 5 died of other causes, while 174 were alive two to three years later. It is probable that all the surviving children had been infected to a greater or less extent, and radiographical examination actually showed the presence of calcified mesenteric glands in 127 of them. It is interesting to note that practically all cases that developed a primary lung lesion died, whereas the majority, which had primary alimentary lesions, survived.

We may conclude tentatively that tuberculous infection in infancy is often liable to cause death, particularly if it occurs by the respiratory tract. Infection is, however, by no means uniformly fatal. Provided the environmental conditions are good, and exposure to infection is not too severe or repeated, it seems probable that latent lesions may result of a type similar to those met with in later years.

Latent Pulmonary Tuberculosis as revealed by X-ray Examination.

In recent years X-ray examination of the chest has come to play an important part in the diagnosis of pulmonary tuberculosis, since it is possible by this means to detect lesions before clinical manifestations have developed. In several countries surveys are now being made to determine the frequency of pulmonary lesions in different classes of the population. Table 99, taken from the report of the Medical Research Council's special survey (Report 1945a), affords an example of the type of result obtained.

It will be seen that between 0.8 and 2.6 per cent. of subjects examined were found to have tuberculous lesions of sufficient severity to demand treatment of some sort. No attention is paid in these figures to healed and often calcified tuberculous foci of the lungs and glands, such as can be found in about 20 per cent. of young adults (see Hetherington *et al.* 1929, Opie 1930, Bradbury 1947).

Natural Antibodies. That the formation of some antibodies is genetically determined and takes place in the absence of antigenic stimulus is established in the case of the antibodies which determine the human blood groups. It is not unreasonable to suppose that in other instances the arrangement of polar forces on the surface of the normal globulin molecule might be such as to make possible to a greater or lesser extent the specific adsorption of antigenic substances. The probability that this will occur is, however, not great, and it is unlikely that such an explanation is generally valid.

Inapparent Infection. A second possibility that accounts for the presence of normal antibodies is that of inapparent infection. A case in point is that of the appearance of diphtheria antitoxin in the blood in the absence of clinically recognizable infection; it is well known that the proportion of Schick-negative individuals increases in succeeding age groups. It is definitely established that the indicated formation of antitoxin is an immunity resulting from inapparent infection, i.e., the carrying of virulent diphtheria bacilli in the throat, and the antigenic stimulus of the presence of small amounts of diphtheria toxin (p. 621). The agglutinins for Flexner dysentery bacilli and some of the common species of *Salmonella* associated with food poisoning not infrequently present in normal human sera to titers of 1.80 to 1.160 in all probability are a consequence of subclinical infections. It is probable that such a sequence of events occurs in a number of diseases and that so-called normal antibodies, such as the neutralizing power of normal adult serum for poliomyelitis virus, measles virus and other infectious agents, are, in fact, immune antibodies. The manner in which a pseudo-racial immunity may be produced through infection has been discussed elsewhere (p. 219).

Common Antigens. As indicated above, however, there are many instances in which it is extremely unlikely that the animal whose serum agglutinates a given bacterium came in contact with it either as a mild infection or otherwise. It is difficult to conceive, for example, of the ordinary cow in the United States coming in contact with the cholera vibrio. In such cases it is not necessary to assume either that the observed antibodies arose *de novo* through some genetic or maturation mechanism or that there was contact with the particular microorganism. It is only necessary to assume that the animal has come in contact with an immunologically similar antigen. The likelihood that this will occur is clearly a function of the frequency with which immunological relationship or identity is shared by diverse organisms and the probability of contact with the unrelated form.

In addition to heterophile antigen, whose apparent random occurrence has been discussed elsewhere, immunologically similar antigens have been found to occur in a variety of seemingly unrelated organisms, and it is probable that many more have not as yet been discovered. For example, cross reactions occur between Type 2 pneumococcus polysaccharide and Type B Friedlander's bacillus, certain species of yeasts, gum acacia and other vegetable gums, and some strains of *Bacterium coli* and *Bacterium aerogenes*, the polysaccharide of Type 14 pneumococcus is immunologically similar to the specific antigen of human blood group A; a constit-

exposed to an unusually high risk of infection (see Heimbeck 1927, 1933, Ross 1930, Hetherington *et al.* 1931, 1935, Herman *et al.* 1932, Shipman and Davis 1933, Geer 1934, Kramer 1934, Stiehm 1935, Jacobs 1938, Soper and Amberson 1939, Hart, Hilton and Morland 1940, Hedvall 1940, Hetherington and Israel 1940, Brahdry 1941, Israel, Hetherington and Ord 1941, Alt, Barth and Day 1941, Hastings and Behu 1941, Lyght 1942). Reference may also be made to similar surveys conducted by X-ray examination, sometimes in association with the tuberculin test, in school children and other classes of the population (see Chadwick 1927, Opie *et al.* 1929, Hewitt and Cutts 1932, Martin *et al.* 1934, Fellows 1934, Rouvillois 1934, Harrington *et al.* 1935, Weber *et al.* 1935, Hetherington, Israel and Kreitz 1938, Pope, Sartwell and Zacks 1939, James 1939, Douglas 1939, Hutchinson and Pope 1940, Galbraith 1941, Trail 1942, Banszky 1942, Clive 1943, Marshall 1945, Bradbury 1947, Umholtz 1948).

Frequency of Tuberculosis in relation to Degree of Exposure to Infection.

It has already been pointed out that in children exposed to contact with patients suffering from open tuberculosis the proportion reacting to tuberculin reaches a high figure in the early years of life (see Fig. 279, p. 1495). Observations by numerous workers on families containing one or more members with a positive sputum have now shown that the children in these families have a much higher morbidity and mortality rate from tuberculosis than children in families with sputum-negative patients, and still more so than children in families completely free from tuberculosis. In this country Cox (1929) analysed the histories of 1,486 children under 5 years of age in Lancashire exposed to contact in the home with open tuberculosis. In these children the death rate from non-pulmonary tuberculosis was 9 times greater in the 0-1 year age group, 14 times greater in the 1-2 age group, and 19 times greater in the 2-5 age group than that of a control child population in the County of Lancaster. The excess deaths, it may be noted, were mainly due to tuberculous meningitis. In households containing one or more persons suffering from tuberculosis but having a negative sputum, the death rate of the exposed children was rather greater than that in control children, but the differences were not significant. Cox's figures received general confirmation from a similar study in Worcestershire (1931).

In the United States even more convincing evidence is available from the extensive observations of Opie (1928-29, 1935) and his colleagues (see Opie and McPhedran 1935, McPhedran and Opie 1935), and of such workers as Ervats, Potter, and Dunn (1934) and Stewart, Gass, Puffer, and Williams (1943). The following figures furnished by Opie, McPhedran, and Putnam (1935), which refer to the development of pulmonary tuberculosis, are of interest in showing not only the much greater frequency of the disease in subjects exposed to open cases of tuberculosis, but also the proportion developing pulmonary tuberculosis in relation to the age of exposure.

(1) Of persons first exposed between 0 and 9 years to contact with sputum-positive patients, 9.92 per cent. of those living 12-14 years after the beginning of exposure had acquired pulmonary tuberculosis, whereas among those exposed to contact with sputum-negative patients, only 1.97 per cent. subsequently developed the disease.

(2) Of persons first exposed between 10 and 14 years to contact with sputum-positive patients, 20 per cent. of those living 10-14 years after the beginning of exposure had acquired pulmonary tuberculosis, whereas none of those exposed to contact with sputum-negative patients had acquired the disease.

(3) Of persons first exposed after 15 years of age to contact with sputum-positive

uent present in peptone is immunologically related to certain streptococcus antigens (group C); the capsule of the anthrax bacillus appears to be immunologically identical with that of *Bacillus mesentericus*; the plague bacillus and paratyphoid B are immunologically related. From these examples²¹ it will be clear that immunologically similar antigens may be distributed in an apparently random fashion in unrelated organisms and it is probable that in some, if not many, instances in which antibodies may be demonstrated for some microorganism with which infection or contact is unlikely, the antibodies are immune rather than "normal," and a consequence of exposure to a similar antigen.

In keeping with this discussion it may be pointed out that there is a strong possibility that there is no such thing as a normal specific antibacterial immunity and that immunity in which a specific antibody may be demonstrated is one that arises as a consequence of exposure to the antigen. The incubation period following injection, for example, provides the opportunity for an immunological response that is difficult to rule out in many instances. In this connection it is of interest that attempts to establish a monoflora of *Bacillus subtilis*, an organism generally regarded as a harmless saprophyte, in bacteriologically sterile rats have resulted in a number of deaths from *B. subtilis* septicemia.

Acquired Immunity. Specific immunity against infection may be of two types. The one, *active immunity*, is due to the direct participation of the host and is gained at the expense of the organism acquiring it. This active immune state, manifested by the presence of antibodies, a heightened cellular reactivity, and a marked increase in resistance to the infection, arises as a consequence of the stimulus of antigen present in the tissues. The antigen may be inadvertently present, as in an attack of disease, or it may be purposely introduced into the tissues in such a form that, while its antigenic qualities are little if at all impaired, disease is not produced. Active immunity may, then, be artificially produced by the parenteral inoculation²² of appropriate antigenic material. In general terms, the methods by which active immunization may be accomplished are:

- (1) T
- (c) in conjunction with protective antiserum,
- (2) the inoculation of attenuated bacteria of greatly reduced virulence for the host;
- (3) the inoculation of bacteria killed by
 - (a) heat or
 - (b) antiseptics;
- (4) the inoculation of bacterial products
 - (a) secreted during life or
 - (b) liberated by the autolysis of dead cells;
- (5) the inoculation of bacteria unrelated to the production of the specific infection.

These may be briefly illustrated.

- (1) Immunity produced by the introduction of living, virulent bacteria

²¹ The earlier literature is reviewed by Ingalls: *Jour. Immunol.*, 1937, 33:123.

²² Oral administration of antigenic material is efficacious only in so far as unmodified antigen is absorbed through the intestinal wall and, compared with parenteral inoculation, is an uncertain and inefficient method.

the lungs from the alimentary tract *via* the lymphatics, the bronchial glands should show the primary, and the lungs the secondary lesions.

From these various sources of evidence we conclude that, with the exception of those cases of miliary tuberculosis in which the lungs have been infected from the blood stream, the vast majority of cases of pulmonary tuberculosis are due to infection by the *respiratory tract*.

Dust and Droplet Infection.

Accepting the inhalation hypothesis, we are faced with the problem of how the bacilli gain entrance to the *respiratory tract*. The evidence brought to bear on this problem is so extensive that we can give nothing more than a brief outline of the conclusions drawn from it. According to Cornet (1889), the chief source of infection is the *dust arising from dried sputum*. Examining 140 specimens of dust taken from various situations—chiefly hospitals, public buildings, and the private rooms of tuberculous patients—he found tubercle bacilli in 40 of them. He believed that tubercle bacilli might remain alive and virulent in dust for 3 or 4 months. He maintained that, apart from his sputum, a patient suffering from pulmonary tuberculosis was harmless. The main objections to this view are: (1) For the tubercle bacilli to be set free by the desiccation of sputum the air must be absolutely dry; if it is at all moist, complete desiccation does not occur. In northern Europe, at any rate, the atmospheric conditions during most of the year are unfavourable for complete desiccation. (2) During the process of drying there is evidence that the tubercle bacilli, especially if exposed to sunlight, are liable to die (Heymann 1901, Mayer, E., 1921, Caldwell 1925, Eidlinow 1927). These objections, however, do not apply to sputum expectorated inside artificially heated buildings where drying may occur quite rapidly.

According to Flugge (1899) and members of his school (Moeller 1899, Heymann 1901, Findel 1907, Ziesché 1907, Hollmann 1924), the tubercle bacilli gain entrance to the *respiratory tract* in the form of *minute droplets* expelled from the mouth and nose of patients suffering from open pulmonary tuberculosis. Buchner showed that bacteria suspended in a very fine spray could be conveyed through several metres of rubber tubing, even when only a light current of air was used. Laschtschenko found that sprayed tuberculous sputum carried tubercle bacilli in suspension over a considerable distance when propelled by an air-current of only 3 mm. per second. By taking suspensions of *Chr. prodigiosum* into his mouth, he showed that organisms were expelled to a slight extent during speaking, to a greater extent in coughing, and to a still greater extent in sneezing. Numerous workers have found that tuberculous patients when coughing expel droplets containing tubercle bacilli; these droplets are projected for varying distances, but not further as a rule than $1\frac{1}{2}$ metres. Guinea-pigs placed about half a metre from the mouth of tuberculous patients, and exposed freely to their cough-spray, not uncommonly contract tuberculosis.

... on the ground that the
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or
over in diameter remained in suspension
for a few minutes, and droplets of only a few μ for a few minutes
expelled in coughing are said to be fairly large, they conclude that primary lung infection
by droplets must be uncommon. Their measurements of the size of the droplets were

is practically identical with the immunity that results from an attack of disease after natural exposure. In experimental work the varying facility with which this mode of immunization can be effected is in part dependent upon the susceptibility of the organism to the particular parasite, and a highly susceptible animal can be immunized in this way only with great difficulty or not at all. The successful use of living cultures involves the administration of small non-fatal doses which are increased as immunity develops.

The relative insusceptibility to infection by some particular route may be taken advantage of, as in Ferran's method, now superseded, for protective vaccination against Asiatic cholera, natural infection occurs via the alimentary tract and subcutaneous injection of the virulent vibrios is followed by a local inflammation but not by a general infection with serious consequences.

The simultaneous administration of virulent microorganisms and protective antiserum provides preformed antibody for combating the invader while allowing the immune reaction of the host to develop. This method is seldom used for the immunization of human beings but is common in some other instances; hogs may be immunized against hog cholera, for example, by simultaneous injection of virus-containing blood and antiserum.

(2) As indicated elsewhere, the virulence of a bacterial culture may be greatly diminished in a variety of ways, such as cultivation under unfavorable environmental conditions and by passage through animal species other than that of the host in question. Perhaps the most familiar examples of the use of such attenuated material are those of smallpox vaccination and inoculation against rabies. In the first instance the passage of smallpox virus in the cow greatly reduces its virulence for man but inoculation with the altered virus of cowpox protects against smallpox infection, and in the second rabies virus from dogs is "fixed," in Pasteur's terminology, or attenuated by serial passage in rabbits. This method is particularly useful in immunization against the filterable virus diseases, since cultures are not available and "killing" destroys the antigenic properties of these agents. (See, however, p. 853.)

(3) Suspensions in physiological salt solution of bacteria killed by heating to 55° to 60° C. for thirty minutes or by treatment with formaldehyde or phenol are widely and successfully used in man against typhoid and paratyphoid fevers and against a variety of microorganisms in the laboratory. Such vaccines are, in general, most useful in infections in which the microorganism does not produce a soluble toxin but contains an endotoxin. This method has the obvious advantage of avoiding all danger of infection while at the same time introducing into the body the substances most intimately connected with the bacterial cell and its activities.

(4) The use of products of the bacterial cell for immunization purposes finds widest application in the case of those organisms which produce soluble toxins. As pointed out above, a solid immunity against diphtheria may be secured by stimulating the production of antitoxin. It is possible to build up such an immunity by using extremely small amounts of unmodified toxin in the early injections with gradual increases as immunity develops. In general, however, it is much more satisfactory to use neutralized or

lung (see Chaussé 1914, 1916, Lange and Nowoselsky 1925, Wells and Lurie 1941, Glover 1944).

There is reason to believe that the smaller the particle inhaled the more likely is this to occur. Wells, Ratcliffe, and Crumb (1948), for example, found that, when rabbits were exposed to a very fine spray of organisms containing nuclei that settled at a rate of less than 1/10th foot per minute, nearly every nuclear particle gave rise to a tubercle in the lung; but when a coarser spray was used, containing nuclei that settled at the rate of about 1 foot per minute, less than 10 per cent. of the nuclear particles gave rise to tubercles. These results cannot, of course, be applied directly to human beings. Apart from the different anatomical configuration of the respiratory tract in the rabbit and in man, it must be remembered that droplet nuclei formed from cough spray are far less liable to contain tubercle bacilli than larger droplets. Moreover it is possible, though at present unproven, that tubercle bacilli in droplet nuclei may be less infective than in fresh droplets, owing to desiccation, exposure to light, or other factors. (See also p 2272)

We are not yet in a position to assess the relative importance of dust, droplets, and droplet nuclei in giving rise to pulmonary infection, nor do we believe that an attempt to do so would be of very much value. In whatever form tubercle bacilli are expelled from the mouth they are potentially infective. The proportion distributed in the atmosphere at any one moment as droplets, droplet nuclei, or dust particles must be determined to a considerable extent by the environmental conditions, and any generalization would probably be subject to so many exceptions as not to be worth making. In the control of tuberculosis the main conclusion is that the patient must be taught to practise a high level of hygiene and not to jeopardize the health of his fellows by spitting or coughing without taking adequate precautions.

Several observers have drawn attention to the risk of aerial dissemination of tubercle bacilli in the post-mortem room (Hedvall 1940, Sloan 1942, Morris 1946, Meade 1948). Sloan, for instance, was able to show that infected droplets were scattered into the air when tuberculous lungs were sectioned.

Endogenous and Exogenous Infection.

The long period during which it was taught that practically everyone reaching adult life had already been infected with the tubercle bacillus was characterized also by the belief that pulmonary tuberculosis of the adult type was the result either of activation of an old pulmonary lesion or of endogenous superinfection. It is now realized that, except in crowded urban communities, a proportion of persons may reach adult life without becoming infected, and more attention is therefore being devoted to the part played by exogenous infection in the pathogenesis of pulmonary tuberculosis.

Terplan (1940) and Medlar (1947, 1948) brought evidence to show that adults who become infected for the first time may develop pulmonary lesions similar to those characteristic of childhood (see Opie below), and that many cases of pulmonary tuberculosis in adults are, in fact, the result of primary infection. The main problem, however, concerns the genesis of pulmonary tuberculosis in those who have already been infected in childhood. Is the disease due to endogenous exacerbation or to exogenous superinfection or re-infection? On this point there is a cleavage of opinion. We have no space here to discuss the evidence in detail; the papers we do quote refer mainly, it may be emphasized, to pulmonary tuberculosis in adolescence and early adult life, not to the late adult type, whose pathogenesis is still very much in doubt.

detoxified toxin. In the first instance a neutral mixture of diphtheria toxin and antitoxin (TAT) is frequently used with satisfactory results; the complex breaks down slowly in the body, liberating free toxin which acts as the antigen. Toxoid prepared by treatment with formalin which has lost its toxicity but retained its antigenicity is generally used at the present time in a partially purified form precipitated by alum. The alum-precipitated toxoid is of some advantage since the toxin-alum complex breaks down slowly in the tissues, liberating toxoid slowly to provide a prolonged antigenic stimulus.

It is difficult to distinguish between bacterial products secreted during life and the products of autolysis; it has been pointed out previously, for example, that usually the soluble toxin titer of a culture rises, not coincident with the phase of active growth, but after cell multiplication slows down and breakdown of the formed cells begins. Studies have been made, however, on the antigenic qualities of autolyzed cultures and of cell extracts, but such antigens are not of great practical value since they are contained, for the most part, in suspensions of killed cells.

(5) Some degree of immunity toward specific infections may be developed by the use of certain kinds of bacteria or bacterial products entirely foreign to the infection in question. In this category, for example, is the undoubted protection conferred against anthrax by the injection of *Bacterium prodigiosum* or *Ps. pyocyanea* or their products. A number of instances of this sort have been described in connection with studies on common antigens and need not be considered further here.

Irrespective of the antigen employed, the animal body requires a period of time to respond with antibody production and for a high degree of immunity successive antigenic stimuli are required. Early injections may consist of killed bacteria, followed by the inoculation of living, attenuated or virulent cells. The process of forcing a very high degree of immunity upon an experimental animal is often termed *hyperimmunization*. If the antigen is injected in such a form that it is slowly absorbed, thus providing a prolonged stimulus, repeated injections may not be necessary. A single injection of alum-precipitated diphtheria toxoid, for example, may give about 80 per cent Schick negatives. Attempts have been made to use *lipovaccines*, suspensions of bacteria in oil, to promote such low absorption, but these are generally unsatisfactory because they are difficult to sterilize.²³

Antibody is presumably first formed in the antibody-producing cells and then spills over into the body fluids and blood stream. It is probable that the lag between inoculation and the appearance of serum antibody is in part due to a mobilization of the processes of synthesis of immune globulin, and in part to the time required for the accumulation of antibody shed by the cells to detectable concentration in the body fluids. Antibody also appears in some of the secretions and excretions. The virus-inactivating agent in the nasal mucus (p. 226) is regarded by some as antibody, and antibody has been found to occur in the feces and urine of immunized animals and man.²⁴

²³ See, however, Halbert, Mudd and Smolens: *Jour. Immunol.*, 1946, 53:291

²⁴ Burrows, Elliott and Havens: *Jour. Inf. Dis.*, 1947, 81:261, Harrison and Banvard: *Science*, 1947, 106:188, Burrows and Havens: *Jour. Inf. Dis.*, 1948, 82:231.

tuberculosis, then far more care should be exercised than has hitherto been general in protecting not only children but adults, even tuberculous adults, against the risk of exposure to infection. There is still a widespread tendency to neglect or minimize the risk of adult infection. In the absence of strong evidence to the contrary, we consider this attitude to be dangerous and reprehensible. Experience of the control of infectious disease, whether of bacterial or virus origin, has established the principle that avoidance of infection constitutes the first line of defence. Vaccination by natural or artificial means plays, as a rule, a much less important rôle. We see no reason why tuberculosis should be regarded as different in this respect from other diseases.

TUBERCULOSIS OF BOVINE ORIGIN. FREQUENCY AND MODE OF INFECTION

Non-pulmonary Tuberculosis.—The incidence of non-pulmonary tuberculosis caused by the bovine type of bacillus varies greatly in different countries and in different parts of the same country, depending in general on the incidence of tuberculosis in cattle and the quantity of milk consumed raw. The figures collected in Great Britain during the second world war, which were based on the examination of over 2,000 strains, showed that about 40 per cent. of cases of non-pulmonary tuberculosis in children under fifteen years of age, about 15 per cent. over this age, and about 25 per cent. of cases at all ages were due to infection with the bovine type (Report 1949, 1952*d*). By applying the proportions of bovine-type infections found in the different forms of non-pulmonary tuberculosis—meningitis, cervical, glandular, abdominal, bone and joint, genito-urinary, and miscellaneous—in the two sexes at different ages to the deaths given by the Registrar-General and to the figures of registered cases under treatment, it was calculated that in the year 1944 there were approximately 14,000 cases of disease and 1,500 deaths due to non-pulmonary infection caused by the bovine type of tubercle bacillus.

In England and Wales about 6.0 per cent. of deaths from all forms of tuberculosis are due to infection of bovine origin. The frequency in other countries is less certain. In the United States, Park and Krumwiede (1910), who examined 436 strains of tubercle bacilli, of which 291 were derived from patients with pulmonary tuberculosis, found that 7.57 per cent. were of the bovine type. This percentage, however, is not applicable to the present incidence of tuberculosis of bovine origin. Since 1917 an intensive campaign has been carried out under the Federal Government to eradicate tuberculosis in cattle, and the extent to which these animals are infected is now on an average probably only about one per cent. of that in Great Britain. Clinical observers are unanimous in agreeing on the comparative infrequency in the United States of cervical and mesenteric glandular tuberculosis in young children, both of which manifestations in this country are generally due to the bovine bacillus (see Reichle 1936). In Germany, Austria, Denmark, Sweden, Poland, and the Netherlands the disease appears to be fairly frequent, though perhaps less so than in Great Britain (see Klimmer 1931, Lange 1932, 1937, Blacklock 1932, Tobiesen *et al.* 1935*a, b*, de Lange and de Bruin 1935, Piasecka-Zeyland 1937, Ruys 1937, van der Hoeden 1937, van der Hoeden and Pannevis 1937, Hedvall 1942, Price 1939, McMurray 1941, Holm 1946*b*).

Source of Infection.—Cattle form the great reservoir of infection with the bovine bacillus. In this country about 30 per cent. of all milch cows react to the tuberculin

The former is of some interest in that it may be associated with immunity to the enteric infections in which the infection is confined to the lumen of the bowel and superficial layers of the intestinal mucosa.

An animal may be simultaneously immunized against more than one antigen with no significant effect on the immune response. Typhoid vaccine, for example, commonly contains typhoid, paratyphoid A and paratyphoid B bacilli (TAB), and other so called polyvalent vaccines containing a variety of microorganisms are effective immunizing agents.²⁵

The immunity produced is variable to some degree and dependent upon the efficacy of the antigen used. Some kinds of bacteria are "good antigens," such as the typhoid bacillus, while others, such as the gonococcus, are "poor antigens" and no effective immunity may be produced. The reason for this is not known. When an active immunity is produced, however, it is generally of long duration and effective over a period of years.

The immune response, as indicated by antibody titer, generally is apparent by the second of a series of injections and the titer increases with succeeding injections until an upper limit is reached. With cessation of inoculations the antibody titer slowly declines and within a few weeks has reached a very low level. Subsequent injection brings about an immediate antibody response, much more rapid and pronounced than that of the initial immunization. It is of some interest that this secondary response may be brought about, although to a lesser degree, by the injection of a heterologous antigen, a phenomenon that has been termed the *anamnestic reaction*: this stimulation to fresh production of one antibody when a new antigen is injected has been an important obstacle to theories of antibody production such as retention of antigen in the cells, etc. It may be noted here that the injection of any antigenic substance stimulates the mobilization of the defense mechanisms of the body, a phenomenon which is made use of in *non-specific protein therapy*, the injection of sterile milk, for example, may markedly stimulate the body to combat invading microorganisms. It will be clear that, under the circumstances, any interpretation of the beneficial effect of vaccine therapy of infectious diseases must be made with caution.

Passive Immunity. In contrast to active immunity, passive immunity involves no active generation of protective substances by the immunized animal. The latter is simply the recipient of antibodies formed in the body of another animal and transferred to the individual to be protected. Such passive transfer occurs in nature from mother to offspring, *in utero* via the placental circulation in man, apes, and rodents in which there is only one layer of cells between the maternal and fetal circulatory systems, and after birth via the colostrum in ruminants in which there are four layers of cells between the two circulatory systems.²⁶ The direct absorption of immunologically unaltered antibody from the lumen of the bowel in the adult guinea pig has been observed also.²⁷

Artificial passive immunization is brought about by the injection of

²⁵ For recommended combinations see Amer. Jour. Pub. Health, 1944, 34 452.

²⁶ See the review in the Lancet, 1941, 1 44.

²⁷ Burrows and Havens Jour. Inf. Dis., 1948, 82 231.

1932, Hedvall 1942). Strong support for this view is afforded by the studies of Sigurdsson (1945) in Denmark.

Investigating the origin of 566 strains of tubercle bacilli from cases of pulmonary tuberculosis typed at the State Serum Institute, Copenhagen, between 1932 and 1940, Sigurdsson found that 91 were of bovine and 475 of human type. Of the bovine strains, 67 came from 165 patients who had been living during the two previous years in rural districts, 11 from 39 patients in urban and rural districts, and 13 from 362 patients in urban districts. Among the 67 purely rural patients, no fewer than 63 had been recently in contact with strongly tuberculous herds of cattle. Milk-borne infection seemed to be improbable, because (a) the clinical features were those of primary lung tuberculosis, (b) the younger children, who drank milk but who were not in close contact with infected cattle, did not develop pulmonary lesions, and (c) pulmonary tuberculosis in adult rural workers who were not living on infected farms but who were exposed to milk-borne infection from these farms was always due to infection with the human type. Further evidence also pointed strongly to the conclusion that pulmonary tuberculosis due to the bovine type of bacillus was associated with close contact with tuberculous cattle and was due to the direct inhalation of tubercle bacilli coughed up by the animals.

Occasionally infection is transmitted from one person to another by contact (Griffith 1938). The clinical course of the disease is often rapid and a considerable proportion of cases prove fatal.

Summarizing, we may say that practically all non-pulmonary cases of bovine origin are contracted through the agency of raw milk or cream. Cheese and butter, it may be noted, probably play an insignificant part in the transmission of infection in Great Britain. Over 80 per cent. of butter is imported and practically all imported butter is made from pasteurized cream. In cheese the tubercle bacillus dies out fairly quickly, and is usually dead before the ripening process is complete. Only in farm butter and in soft cheese, i.e., unripened cheese eaten within a few days of its preparation, are tubercle bacilli likely to be found alive. Pulmonary tuberculosis of bovine origin is mainly a rural disease, and infection appears to be contracted more often by the aerogenous than by the alimentary route.

It is of interest to recall the changes that our views on tuberculosis of bovine origin have undergone in the present century. At the London Congress on Tuberculosis in 1901, Koch asserted, on the basis of inadequate experimentation, that bovine bacilli were virtually non-pathogenic to human beings. His views were attacked by M'Fadyean (1901) and by Ravenel (1901) on the basis of strong, though circumstantial, evidence. As the result of considerable opposition, Koch modified his views at the Washington Congress in 1908, to the extent of admitting that human beings might be infected with bovine tubercle bacilli, though he maintained that serious disease due to these organisms was very rare, and that preventive measures to protect the human population against tuberculosis of bovine origin were quite unnecessary. The extensive labours of the Royal Commission on Tuberculosis in this country, and of continental and American workers, revealed the fallacy of Koch's teaching, and showed that non-pulmonary tuberculosis in childhood was frequently caused by the bovine bacillus. The subsequent work of Griffith, Munro, Lange, and others drew attention to the occurrence of bovine bacilli in pulmonary tuberculosis; and infections of bovine origin as a whole must be regarded as a serious menace to the human population in those countries where tuberculosis of cattle is common. The evidence suggests that bovine bacillus is quite as virulent for man as the human bacillus, and pos-

immune sera such as the antitoxic sera of horses immunized against diphtheria toxin, tetanus toxin and similar antigens, or of convalescent sera taken from recovered human cases of the disease in question. Passive immunization is most effective with antitoxic sera; with exceptions such as virus neutralizing sera, antipneumococcus sera and the like, antibacterial sera are generally not highly effective either prophylactically or therapeutically.

Unlike active immunity, passive immunity is not of long duration, generally not more than two or three weeks. Repeated injections of tetanus antitoxin, for example, must be made at weekly intervals as long as the danger of infection remains. Horse serum globulin acts as a foreign protein in the human body and tends to be eliminated as such. Passive immunization with homologous serum is possibly of somewhat longer duration.

HYPERSENSITIVITY

The response of the animal body to the presence of antigen in the tissues is clearly an advantage when the antigen is a toxin or the cell substance of a pathogenic microorganism. It was early apparent, however, that an initial

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and is most striking when the antigen is initially bland. While it may play some part in effective immunity, there is also reason to believe that hypersensitivity to the cell substance of bacteria may, in some instances, contribute to the pathology of the disease resulting from infection as, for instance, in acute rheumatism and arthritis, and hypersensitivity to non-living antigens may also result in disease of non-infectious nature. Hypersensitivity is quite general and may take a variety of forms in both experimental animals and man.

Anaphylaxis.²⁸ The sensitizing effect of an initial inoculation of antigen was observed by Richer in 1902 in a study of the immunization of dogs with toxic extracts of the sea anemone. About this same time Theobald Smith noted the lethal effects of second and third inoculations of diphtheria toxin-antitoxin in the guinea pig when the inoculations were widely spaced, and in 1905 Otto found that the active agent in the mixture was horse serum. This was subsequently studied in considerable detail by Rosenau and Anderson and the general picture is now quite clear.

If a guinea pig is inoculated with horse serum at intervals of perhaps four to eight days, it responds with the development of an immunity and precipitating antibody appears in the serum. If, however, an interval of ten to twelve days elapses between the first and second inoculations, the animal becomes hypersensitive as a consequence of the first inoculation and the second is highly toxic. The state of hypersensitivity so induced by the sensi-
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method of examination was recommended by Gougerot (1945). When examining the *cerebrospinal fluid*, it is best to allow the fluid to stand till a fibrinous clot forms; this should be removed with a platinum loop, spread on a slide, and stained in the usual manner for acid-fast bacilli. Otherwise it should be centrifuged and treated like urine. In *pleural fluid*, except when it is purulent, tubercle bacilli are so scanty that recourse should be had to culture or animal inoculation. In adult patients without sputum or in children who swallow it, microscopical examination may be made of the *fasting stomach contents* or of the *faeces*, but cultural methods are much superior.

In *pus* the bacilli are often very difficult to demonstrate; Gardner (1926), following Spengler (1907), recommends the use of half-saturated aqueous picric acid as a counter stain.

The microscopical method of examination, though very simple, must not be undertaken without due regard to the errors of the technique employed. Microscopical examination alone cannot distinguish between tubercle bacilli and other types of acid-fast bacilli. Besides saprophytic acid-fast bacilli of the *Mycobacterium* group, which are widespread in hay, straw, dust, animal litter, soil, and water, there are a number of other organisms, such as the leprosy bacillus, John's bacillus, and some species of *Actinomyces*, which are acid-fast and which may occasionally give rise to confusion.

In order to avoid contamination of the material with these organisms, strict attention must be paid to the preparation of the actual films. In particular, all stains and reagents should be made up with fresh glass-distilled water; and all glassware should be soaked in 50 per cent. nitric acid for at least 15 minutes. Only new slides should be employed, since there is no simple method of removing acid-fast bacilli from slides on to which they have been fixed and stained, and every slide should be thoroughly flamed in a Bunsen burner immediately before use. If the material has to be rubbed up in a drop of water on the slide fresh glass-distilled water or water from a hot tap in constant use should be used, since acid-fast bacilli are usually abundant in cold-water taps (see Chapter 16). Care must be taken to avoid contamination of the cedar-wood oil reservoir from the surface of a previous film, and the oil-immersion objective should always be cleaned after examining a positive film. All apparatus, particularly if made of metal or rubber, used in the collection of catheter and other specimens, should be kept under conditions that will prevent the growth of saprophytic acid-fast bacilli on their surface. For references to these and other sources of error see G. S. Wilson (1933), Saenz and Costil (1936), A. H. Wells (1936), Tytler, Edwards and Trayer (1938), Hauduroy (1944), Tytler (1945), and Report (1945b).

When all these precautions have been taken, the importance to be attached to the finding of acid-fast bacilli will vary with the nature of the material examined. Though acid-fast bacilli in sputum or cerebrospinal fluid may be regarded as almost certainly diagnostic of tuberculosis, their presence in *faeces* or urine must be interpreted with far greater caution, since the presence of saprophytic acid-fast, including smegma, bacilli cannot with certainty be excluded. In blood so many artefacts are liable to be present that it is extremely doubtful whether the search for acid-fast bacilli is justified at all.

Of recent years *fluorescence microscopy* has been used for the examination of tubercle bacilli. The method depends on the observation that tubercle bacilli, stained with a suitable dye and irradiated with ultraviolet light, transform the invisible short waves to longer visible rays. Hagemann (1937a, b), who introduced the method, at first advocated berberine sulphate as the dye, but later he (1938) recommended auramine. Observed through a yellow filter in the eyepiece, the fluorescent organisms appear as golden yellow

anaphylaxis is a laboratory artifact in that it results from the purposeful inoculation of antigen into the tissues, and shock is produced by the rapid inoculation of relatively large amounts of antigen, usually directly into the blood stream. It is artificial in that it occurs only under conditions set up by man, in experimental inoculation of animals and in serum therapy in man.

The sensitizing dose may be exceedingly small, as in the case of horse serum in the guinea pig, and varies with different experimental animals. The shocking dose is usually 0.01 ml. of horse serum for the guinea pig. Within a certain range the severity of the shock produced is directly related to the size of the shocking dose, i.e., mild shock is produced by a small dose and lethal shock by a larger dose. The route of inoculation of the sensitizing dose is not important. For some animals and some antigens several sensitizing doses may be required to develop hypersensitivity, but in any case the doses must be small and widely spaced. A single dose suffices to shock and the route of inoculation is important. Intravenous and intracardial inoculation is most effective, and in the guinea pig, which is the most readily sensitized and shocked of the usual experimental animals, intraperitoneal inoculation of the shocking dose is effective but the reaction is delayed and larger doses are required. Once established, the anaphylactic state persists more or less indefinitely but diminishes appreciably in the guinea pig after a few weeks. If the shock produced is not lethal, the animal is temporarily refractory, that is to say, is *desensitized*, but hypersensitivity reappears, in the case of the guinea pig after perhaps two weeks or less. Guinea pigs, rabbits and dogs are the common experimental animals, but birds may be sensitized, while mice, rats and monkeys can be sensitized only with difficulty and then irregularly. The symptoms and postmortem pathology are essentially identical within a given animal species regardless of the antigen used, but differ from one animal species to another.

The Guinea Pig. Following inoculation of the shocking dose the animal remains quiet for a few moments but very soon becomes *restless*, the fur becomes *ruffled*, and the animal *rubbs its nose* and *sneezes*. *Clonic and tonic convulsions* set in, the animal falls to its side, *respiration becomes labored* and the animal *dies gasping for breath while the heart continues to beat*. This symptom complex is produced in varying degree in non-fatal shock. The outstanding postmortem findings are the marked distention of the lungs and hemorrhages on the under side of the diaphragm. The lungs remain distended after the thoracic cavity is opened and when removed from the body and cut. The bronchioles are sharply constricted with the retention of air in the alveoli, and the immediate cause of death is suffocation. The contraction of the bronchioles is not prevented by vagotomy or curare and is muscular or immediately neuromuscular in origin.

The Rabbit. The rabbit is less susceptible to anaphylactic shock than the guinea pig but fatal shock may be produced by intravenous inoculation of relatively large amounts of antigen. The symptoms and pathology differ sharply from those in the guinea pig, especially in the degree of difficulty in respiration. The animal falls on its side, shows *convulsive movements* and *passage of*

which have been cultivated are really tubercle bacilli or are derived from the material under examination. Though numerous methods have been suggested for distinguishing between tubercle and saprophytic acid-fast bacilli (see Collins 1953, Engbaek 1954), the truth remains that, in primary diagnosis, no organism should be reported other than provisionally as a tubercle bacillus until it has been finally identified by animal inoculation.

The microscopical demonstration of acid-fast bacilli in the fasting stomach contents was described originally by Meunier in 1898, but was not adopted as a routine till it was rediscovered by Armand-Delille (1927) nearly thirty years later. Cultural examination has been found to be considerably more delicate than the microscopical method and is now widely used (see Webster 1941, 1943, Davies and Doherty 1942, Robinson and Dunn 1943). It is particularly suitable for hospital patients. For other patients the laryngeal swab method is generally preferable (see Report 1945b, Forbes *et al.* 1948). This is a development of the technique described by Behmann (1930) and Wood (1936). It is not quite so sensitive as gastric lavage, but this defect is counterbalanced by the fact that it causes much less inconvenience to the patient and can therefore be repeated more often. Both stomach contents and laryngeal swabs should be examined as soon after collection as possible. For the examination of faeces some concentration method, such as the ligroin method, should be employed. Cultivation of faeces is of help in the diagnosis of intestinal tuberculosis and, as an alternative to gastric lavage or laryngeal swabs, in persons from whom sputum is absent or unobtainable. According to Tytler (1915), if carried out carefully, it is only slightly less sensitive than the culture of stomach contents (see also Mishulow *et al.* 1934). Wagener and Reuss (1953) recommend the quaternary ammonium detergent Bradisol, to which tubercle bacilli are resistant. By its use they were able to demonstrate tubercle bacilli not only in urine and faeces but also in town sewage.

Pleural fluid may be prevented from clotting by the addition of two drops of 20 per cent. sodium citrate solution for every 10 ml. of fluid; it is then centrifuged and the deposit is planted with a pipette on to suitable egg media. Alternatively, the fluid may be distributed into bijou bottles in 10 ml. amounts and allowed to clot; the clots are then spread thoroughly over the surface of egg tubes (Close 1946).

Between the two world wars, mainly owing to the assertion of Löwenstein that tubercle bacilli could be isolated from the blood of a high proportion of patients suffering not only from pulmonary and non-pulmonary tuberculosis, but also from such diseases as articular rheumatism, polyarthritis, chorea, multiple sclerosis, schizophrenia, and retro-bulbar neuritis, considerable attention was paid to the subject of tuberculous bacteraemia. We do not propose to discuss this work in detail. Though tubercle bacilli may be demonstrated by cultural and animal inoculation methods during life in 5-10 per cent. of severe, advanced and progressive cases of pulmonary tuberculosis and 30-40 per cent. of cases of miliary and meningeal tuberculosis, and in 50 per cent. of fatal tuberculous cases examined *post mortem*, they can very rarely be found in the early stages of pulmonary or non-pulmonary tuberculosis. A search for these organisms in the blood is therefore of little value in diagnosis. The great majority of workers entirely failed to substantiate Löwenstein's findings, which were almost certainly due to gross errors in technique. The whole subject was critically reviewed by G. S. Wilson (1933). See also Report (1935c).

Serological Methods in Diagnosis.—Serological methods of diagnosis were described in earlier editions of this book but, as they are no longer in vogue and as they have no special academic interest, there is no justification for doing more than merely refer to them here (see 3rd edition 1946, p. 1323). The agglutination test was unreliable. The complement-fixation test, though positive in 40-90 per cent. of established cases of pulmonary and non-pulmonary tuberculosis, was often negative in the early stages of the disease when diagnosis was most in doubt. The precipitin test was found to be of little or no practical help.

feces and urine. Respiratory movements may continue briefly after the heart has ceased to beat. On autopsy the pulmonary dilatation found in the guinea pig is absent and the most conspicuous and characteristic feature is the extreme dilatation of the right side of the heart. The immediate cause of death is heart failure resulting from constriction of the branches of the pulmonary artery and consequent dilatation.

The Dog. Anaphylactic shock in the dog occurs in two stages. The first is characterized by restlessness, the animal vomits and passes urine and feces, and collapses with signs of extreme muscular weakness and respiration is labored. The blood pressure falls rapidly as a consequence of capillary dilatation and stasis in the tissues. In non-fatal shock recovery from this stage is rapid, but in fatal shock the weakness is progressive, vomiting and diarrhea continue, convulsions may occur and the animal becomes comatose and dies. On autopsy the outstanding feature is the congestion of the viscera and the pronounced distention and congestion of the liver, which is a consequence of constriction of the hepatic veins and injury to the liver cells resulting in edema from the transudation of fluid.

In addition to the characteristic phenomena noted above, anaphylactic shock also has common features which may be more or less pronounced in different animal species. Fall in body temperature is usual and leucopenia occurs which results from aggregation of leucocytes in the capillaries. Coagulation time of the blood is increased, usually most marked in the dog, as a result of the liberation of heparin rather than direct interference with the clotting mechanism, and serum complement is reduced. Congestion and hemorrhage of the gastro-intestinal tract are common postmortem features.

Local Anaphylaxis. Local occurrence of anaphylaxis was observed by Arthus in 1903 and is called the *Arthus phenomenon*. If rabbits are injected repeatedly with antigen such as horse serum, a local reaction appears which becomes more and more intense as the inoculations are repeated. The site of inoculation first shows a transient swelling, but later the swelling and edema persist and progress to induration and localized necrosis. The local phenomenon is a result of a general sensitization since later inoculations need not be in the same site as earlier ones. The Arthus phenomenon appears to be confined to the rabbit among experimental animals, or at least is very difficult to produce in other animals, but occurs with some frequency in man, as in protracted serum therapy with successive intramuscular inoculations of antiserum, or in prolonged series of inoculations as in the earlier Pasteur treatment for rabies.

Passive Anaphylaxis. Anaphylactic hypersensitivity is passively transferable from sensitized female guinea pigs to offspring, and by inoculation with serum from either sensitized or immunized animals. It can be produced with a high degree of consistency by inoculation of guinea pigs with immune rabbit serum, and in general the sensitizing effect is proportional to the precipitin content of the antiserum. The recipient is not immediately sensitized following the injection of antiserum and usually, though not invariably, a period of some hours elapses before sensitivity appears. If antigen is injected first, followed by the inoculation of antiserum, reversed passive anaphylaxis occurs, also usually some hours after the inoculation of serum.

example, of 468 tuberculous patients investigated by Furcolow, Hewell and Nelson (1912), 99.6 per cent. reacted to 0.0001 mgm. or less of a particular batch of P.P.D., and some reacted even to 0.000000001 mgm. On the other hand, it was found that patients who were seriously ill, who had a short history of the pneumonic form of disease, and who were apparently resisting badly, had a lower degree of sensitivity. In confirmation of this last observation, Woodruff (1918) found that, in pulmonary tuberculosis patients, the number of organisms in the sputum varied inversely with the degree of sensitivity to tuberculin. Turner (1953), who followed up 191 treated cases of pulmonary tuberculosis, observed that relapse was commonest among those with the highest and those with the lowest degree of allergy.

It may be noted that in childhood a positive reaction may become negative with complete healing of the lesion, though the frequency with which this occurs is not known (see Lloyd and Macpherson 1933, Opie 1935, Zacks and Sartwell 1912). Dahlstrom's (1910) conclusion, however, that a high proportion of weak reactors in children become negative with the passage of years is very questionable; it has been criticized on the ground that the dilution of tuberculin he used was so low that many of the reactions he obtained were probably non-specific (Furcolow *et al.* 1911). Most observers would agree with Hardy's (1916) conclusion that tuberculin sensitivity tends to persist for a long time, even in the absence of reinfection. The allergic condition is often maintained by the persistence of tubercle bacilli in quiescent or healed lesions. According to Opie and Aronson (1927) tubercle bacilli are frequently detectable by guinea-pig inoculation even in apparently normal lung tissue, though both Feldman and Baggenstoss (1938, 1939) and Saenz and Canetti (1938) doubt this. Howe (1938) has recorded rapid variations in the sensitivity of both tuberculous and non-tuberculous persons to tuberculins, associated to some extent with changes in blood pressure. It is generally assumed that a positive tuberculin reaction precedes or accompanies the appearance of recognizable lesions by X-ray examination, but Levine (1939) brought evidence to show that in infants under one year of age the reverse sequence may be noted. This may perhaps be due to the poorer antibody response in early infancy than in later years. The comparative liability of tuberculin-positive and tuberculin-negative reactors to develop pulmonary tuberculosis will be considered later (pp. 1522-5).

A *delayed tuberculin reaction* was described by Daniels (1943), developing several weeks or months after the intradermal injection of old tuberculin. It is regarded as an indication that the person has become infected with tuberculosis some time after the test. The reaction is apparently due to the persistence of tuberculin in the skin. If this interpretation is correct, the delayed reaction may serve as a useful indicator of primary infection.

Animal Inoculation Tests in Diagnosis.—The most delicate test for tubercle bacilli is animal injection, and the most suitable animal is the guinea-pig. The susceptibility of the guinea-pig is extremely high; even minute amounts of infective material will render this animal tuberculous. The material—sputum, pus, milk, etc.—should be injected subcutaneously or intramuscularly into the thigh; the advantage of intramuscular injection is that the local abscess which forms does not ulcerate through the skin. It is wise to inject at least two animals, in case one dies of secondary infection—an occurrence which is very common after the inoculation of urine or faeces. One animal should be killed 3-4 weeks later, and if no signs of tuberculosis are apparent, the other should be kept for 6-8 weeks after inoculation before being killed.

Function of Haptenes in Anaphylaxis. The reaction may be, and probably is, a general sensitization can be accounted for in a few instances simple compounds, including arsphenamine, picryl chloride and 2, 4-dinitrochlorobenzene, have been shown to sensitize guinea pigs and sensitization to arsphenamine has been observed in man. Haptenes will usually not elicit shock, though some of the larger molecules such as azo dyes will do so, but the inoculation of haptenes specifically prevents shock by inoculation of the complete antigen. The role of haptenes in specificity is further shown by the use of conjugated antigens containing the same hapten but different protein. A conjugate of a hapten and a specific antigenic globulin can be shocked by the same antigen.

Anaphylactic Shock in Guinea Pigs. In the foregoing discussion that the occurrence common to anaphylactic shock in different animals is a contraction of the smooth muscle, predominantly in the bronchioles in the guinea pig, in the pulmonary artery in the rabbit, and in the hepatic veins in the dog, and in all three the gastro intestinal tract is affected. It is, then, of considerable importance to study the mechanism of the abrupt contraction by Schultz in 1902, and by Schultz in 1903, with uterine horn.

The uterine strip is suspended in a bath of Ringer's solution with one end tied fast and the other attached to a kymograph, and the contractions are recorded when antigen is added to the bath. The reaction is exquisitely sensitive, a sensitized strip giving typical contraction in final dilutions of horse serum antigen as high as 1:1000 million, and sharply specific. All of the essential features of anaphylaxis may be reproduced *in vitro* by this method, including active or passive sensitization of the uterine strip *in vivo*, passive sensitization *in vitro*, specific desensitization, etc. It is significant too that uterine horn from immunized as well as sensitized guinea pigs will respond to antigen with shock.

Mechanism of Anaphylactic Shock. It will be clear that anaphylaxis rests on an immunological basis and that shock is the result of an antigen-antibody reaction. There is good reason to believe that the union is one of antigen with intracellular or sessile antibody. For instance, as just indicated, sensitization and shock may be produced in isolated tissue, and the incubation period in passive sensitization is regarded as necessary for the taking up of inoculated antibody by the cells. Desensitization consists of saturating, or nearly saturating, sessile antibody, and the protective effect of immunity is a prevention of antigen reaching intracellular antibody by union with serum antibody. While by no means final, these interpretations are generally accepted. The means by which shock is produced through antigen-antibody union is not so clear and two general theories have been proposed.

One of these is the *anaphylatoxin theory* which is based on the occurrence of *anaphylactoid reactions* produced by the inoculation of normal serum made toxic by treatment with kaolin, starch and other similar adsorbing agents. On inoculation such toxified sera give a reaction which is closely similar to, but not identical with, anaphylactic shock. Furthermore, peptone

subcutaneously into the opposite thigh with a pure culture of tubercle bacilli, the slight local lesion heals rapidly, but no nodule forms. Instead, on the 1st or 2nd day after inoculation, a circular dark-coloured area of induration, about $\frac{1}{2}$ to 1 cm. in diameter, appears at the site of injection. The next day the skin over this ecchymotic area undergoes necrosis; and later it is thrown off leaving a shallow ulcer, which heals quickly; the focal lymphatic glands remain unaffected. This reaction was first observed by Koch (1891a), and is known as Koch's phenomenon. For its success three conditions are necessary (Löwenstein 1913): (1) the primary infection must be a slight one, so that the disease runs a chronic course; (2) the re-infecting dose must be given as late as possible after the sensitizing dose; the longer the animal has been infected the higher is its resistance; (3) the re-infecting dose must not be too large.

It is clear that the reaction of a tuberculous guinea-pig to a fresh infection is different from that of a normal guinea-pig to a primary infection. Some experiments conducted by Debré and Bonnet (1922) will make this clearer.

A. One group of guinea-pigs was injected with 0.1 mgm. of tubercle bacilli every day for 5 days. *Result.*—Five nodules appeared, identical in their period of incubation (8 to 10 days), in their evolution, and in their accompanying glandular reaction. *B.* Another group of guinea-pigs was injected with 0.1 mgm. of tubercle bacilli every 5 days on five successive occasions. *Result.*—The first two nodules and their accompanying glandular reaction were identical with those in Series *A*. The third nodule appeared after a short incubation period than the first two, was much smaller—the size of a lentil instead of nut—did not ulcerate, and caused only a slight glandular swelling. The fourth and fifth nodules were identical with the third. *C.* Another group of guinea-pigs was injected with 0.1 mgm. of tubercle bacilli every 10 days on five successive occasions. *Result.*—The first nodule and its accompanying glandular reaction were similar to those in Series *A*. The second and third nodules resembled the 3rd, 4th, and 5th nodules of Series *B*. The fourth nodule produced either a typical Koch's phenomenon, or rapid abscess formation followed by cicatrization, without any glandular enlargement. The fifth nodule constantly provoked the typical eschar described by Koch. With these particular doses, it will be seen that Koch's phenomenon did not appear till about 6 weeks after the first injection. The main lesson to be learnt from this experiment is that the "clinical" superinfection of a tuberculous guinea-pig is impracticable after the appearance of the local abscess. Before the local lesion has appeared the animal reacts to a fresh infection just as a normal animal; but once the local lesion due to the first injection has developed—generally in 8 to 10 days—the lesions produced by subsequent injections become less and less, till after 6 weeks the freshly injected organisms call forth an acute reaction, quite unlike the chronic process that follows a primary infection.

These observations are similar to those made on experimental syphilis, which have shown that it is possible to produce in the ape a series of chancres, so long as the second injections are made before the appearance of the primary chancre; once the primary chancre has appeared, further inoculation of syphilitic material fails to produce a fresh chancre. The guinea-pig that has developed a local tuberculous lesion, or the ape that has developed a primary chancre, reacts differently from a normal animal to a fresh infection. As we noted in Chapter 51, the tuberculous guinea-pig is in fact hypersensitive; and, so to speak, is at once more sensitive and more resistant to the infective material than the normal animal. Whether the immunity is only a symptom-immunity, or whether it is sufficiently high to prevent the actual invasion of the organisms at the site of the second

shock, produced by the inoculation of peptone, is also very similar. The theory, originally based on the assumption that the causative antigen-antibody reaction occurs in the blood stream but not completely inconsistent

accepted.

The other theory, which has gained wide acceptance, rests on the assumption that the intracellular union of antigen and antibody results in the liberation of histamine. There is a good deal of indirect evidence in support of the role of histamine in anaphylactic shock. For example, the reaction produced by the inoculation of histamine is indistinguishable from anaphylactic shock. There is no reason to believe that antibody is fixed primarily or even predominantly in smooth muscle cells, but there is a high correlation between histamine sensitivity and the cells concerned in anaphylactic shock. Furthermore, the tissues affected in anaphylactic shock are those that show the highest content of histamine; there is some evidence which suggests that leucocytes are a source of histamine and, as indicated above, they aggregate in affected tissues in shock. In addition many, though not all, substances showing antihistamine activity appreciably or markedly modify anaphylactic shock; of these epinephrine and ephedrine are among the most effective. On the whole, it seems probable that histamine plays an important part in anaphylactic shock, and there is some evidence that choline or acetylcholine may have some minor significance. However, the basic question, in what manner histamine is liberated as a consequence of the union of antigen with sessile antibody, still remains unanswered.

Serum Sickness. The inoculation of man with antiserum, usually from the horse, produces in some persons a characteristic syndrome termed serum sickness. The symptoms include rash, often urticarial in nature, fever, joint pains, some edema, and swelling of the lymph glands regional to the site of inoculation, in combinations and emphasis that are variable from one individual to another. This reaction is to be distinguished from the febrile and local reactions commonly following the inoculation of foreign protein and is most commonly attributable to a hypersensitivity to horse serum irrespective of its antibody content, though essentially similar reactions may be produced following the inoculation of toxoids, vaccines, etc., as a consequence of hypersensitivity to constituent antigen. The incubation period may be as short as two hours or as long as twenty-four days, and most often is eight to twelve days. The reaction may follow initial inoculation of horse serum, but more frequently there is a history of prior inoculation. It is specifically antagonized by epinephrine, ephedrine, etc., and, like anaphylactic shock, is presumably a consequence of the sudden liberation of histamine in toxic amounts.

The hypersensitive individual gives an immediate reaction, i.e., appearing within ten to twenty minutes, to the intradermal inoculation of 0.1 ml. of 1:10 dilution of horse serum (a similar test for sensitivity to diphtheria

be tested with the international standard (see Chapter 13). Graded dilutions of the standard and test preparations are injected intracutaneously into sensitized guinea pigs (see, e.g., Okell and Parish 1927, Parish 1935).

This method, originally described by Römer (1909), consists in inoculating the tuberculin into the animal's flank. As many as 12 or 16 injections can be made on the depilated skin of the same animal. Twenty-four hours after injection a large papule is seen with a haemorrhagic necrotic centre—the so-called cockade reaction. The reaction reaches its height after 48 hours, and later necrosis becomes more evident. Slight infiltration and swelling cannot be regarded as positive. Römer's test is positive about 3 weeks after infection. Dilutions of the tuberculin to be tested are compared with the standard tuberculin, and the relative potencies of the two products determined. A more refined method is described by Long, Miles and Parry (1934).

Tuberculo protein preparations may be standardized against old tuberculin, either by using the guinea pig method or by making titrations on human subjects (see Lichtenstein 1931, Appel *et al.* 1931, Barnwell and Pollard 1931, Aronson 1931, Long 1931, Long *et al.* 1931); or against the international standard PPD (Mammalian).

The Reaction to Tuberculin.—Koch found that 2 ml. of old tuberculin injected subcutaneously into a normal guinea-pig had very little effect on it; but the injection of as small a quantity as 0.01 ml. into a tuberculous guinea-pig—infected 8 to 10 weeks previously—killed it within a few hours. *Post mortem*, the local lesion at the site of the tuberculin injection was very congested, and often dark or almost violet in colour, the focal glands were also very congested; the liver and spleen, besides their tuberculous lesions, showed on the surface numerous punctiform dark red spots looking like ecchymoses, but which microscopically were found to consist of enormously distended capillaries in the neighbourhood of the tuberculous foci, filled with red blood corpuscles. That is to say, the effect of the tuberculin was to produce (1) a local lesion, at the site of injection; (2) a focal lesion, around the tubercles in the tissues, and (3) a constitutional reaction, terminating in death (see also p. 1329).

Analogous observations were made on man. A new-born infant, which has never come into contact with tuberculosis, can withstand the injection of 1 ml. of old tuberculin. A healthy adult, who has been infected, but who is not suffering from clinical tuberculosis, can withstand the injection of about 0.01 ml. without suffering from more than transient malaise and slight pain in the limbs. But the same quantity injected into a clinically tuberculous patient gives rise to a severe reaction, characterized by malaise, pains in the limbs, cough, dyspnoea, rigor, vomiting, and a high fever; the temperature begins to rise about 4 hours after the injection, reaches a maximum of 102° to 101° F., and passes off in 12 to 15 hours. As in the guinea-pig, the reaction is (1) local—an inflammatory reaction occurs at the site of injection (*Stichreaktion*); (2) focal—acute congestion occurs around tuberculous foci; this can be actually observed in lupus patients; an injection causes redness and swelling of the lesion, lasting for 2 or 3 days, followed by the formation of crusts of dried exuded serum, which drop off in 2 or 3 weeks leaving a clean red scar; (3) a constitutional reaction, already described.

The tuberculin reaction can be obtained not only in animals actually infected with the tubercle bacillus, but also in animals that have been injected with dead tubercle bacilli. Bessau (1916), for example, was able by the intraperitoneal

toxoid is the Moloney test, p. 618), or the instillation of a drop of horse serum in the conjunctival sac. In the first instance an irregular wheal surrounded by an erythematous zone constitutes a positive reaction, and in the second a diffuse conjunctivitis appears in the hypersensitive individual. Desensitization may be effected by the inoculation of very small amounts of serum, usually doubled in successive inoculations, over a period of time; the inoculations may be given at intervals of fifteen to twenty minutes because of the rapidity with which the reaction occurs.

The question may be raised as to why serum sickness rather than anaphylactic shock occurs in the hypersensitive individual. In general there seems to be little tendency in man to generalized shock in this and other manifestations of hypersensitivity and, as in dogs and monkeys, there is a tendency to localization of the manifestations of the reaction to the respiratory tract, the gastro-intestinal tract and the skin. A number of instances of typical anaphylactic shock have been reported in man though it is rare, and when generalized shock occurs it is often fatal.

Allergy. Serum sickness is perhaps best regarded as a form of anaphylaxis in man, but only a small part of the manifestations of hypersensitivity are those of serum sickness, and the remainder may be grouped under the general head of allergy. Like anaphylactic shock, an allergic reaction is a consequence of the union of antigen with sessile antibody. The antigen is often designated allergen and the corresponding antibody reagin because it was thought earlier that they differed from antigen and antibody. It is now generally recognized that there are no essential differences but the terms persist even though a number of workers have urged that they be dropped.

There are a number of differences between anaphylaxis and allergy which are rather of degree than of kind, but which in the aggregate tend to distinguish them. Thus, allergy is naturally acquired while anaphylaxis is artificially produced, allergy is often a systemic reaction while anaphylaxis is only rarely so, allergy is often associated with

muscle contraction a

inheritance of a predisposition to at least some forms of allergy is an important, perhaps often a determining, factor. The constitutional factor is a predisposition only and contact with the antigen is essential to the development of the allergic state. Inheritance in man is frequently difficult to demonstrate, but detailed studies on familial association have made it highly probable that predisposition to allergic hypersensitivity is genetically determined. It has been suggested²² that the predisposition is determined by a pair of allelomorphic genes, H determining non allergy and h allergy. The possible genotypes are HHH or pure normal, hh determining allergy which develops before puberty, and Hh the normal transmitter in which allergy may develop after puberty. In confirmation of observations on man, the inheritance of predisposition to

²² Wiener, Zieve and Fries. *Ann Eugenics*, 1936, 7, 141.

Sensitivity to tuberculo-protein appears to differ in other ways from that due to tuberculin. Thus, guinea-pigs sensitized to tuberculo-protein are not immune (Seibert 1933), whereas some degree of immunity invariably accompanies sensitivity to tuberculin. Moreover, by tissue culture it can be shown that in tuberculin sensitivity the body cells are injured when brought into contact with tuberculin (Rich and Lewis 1927-8, Aronson 1931), whereas there is so far no evidence to show that tuberculo-protein is harmful to the cells of non-tuberculous animals. It may be noted that Brahic and Veyron (1939) found a striking parallelism in man between the reaction to tuberculin injected into the skin and that to histamine (see p 1310). Patients giving a strong tuberculin reaction likewise reacted strongly to histamine, and those with a weak tuberculin reaction gave only a feeble histamine reaction.

These observations are consistent with the view that the type of antibody associated with tuberculin sensitivity differs from that associated with anaphylaxis. The tuberculin type of antibody is apparently so strongly fixed to the cells that its presence in the circulation, if it does occur, is almost impossible to demonstrate. On the other hand, the type of antibody associated with anaphylaxis is the usual precipitin type of antibody. (See Chapter 51.)

The Relation between Allergy and Immunity

Of recent years, owing partly to the observations of Heimbeck and his Scandinavian colleagues on the development of tuberculosis in nurses and students, and partly to the observations on animals of Rich and his colleagues in the United States, the relation between allergy and immunity has been under active discussion. Two opposing views have been expressed, each based on circumstantial evidence. It must be our endeavour to present the two sides of the case as fairly as we can.

In the first place we must define our terms, because some confusion has arisen from lack of clarity in this respect. Rich, for example, defines allergy and immunity mainly in terms of results. If allergy is defined as a state in which the tissues are damaged and immunity as a state in which they are protected, then clearly allergy must be bad and immunity good. In so far as bacteriology is concerned, we would rather define allergy as a condition which may develop as the result of introducing bacteria or their products into the body, and which manifests itself as a heightened or accelerated inflammatory response towards a particular type of antigen or other related substance, irrespective of the balance of harm or benefit that the altered response confers on the allergic host. Acquired bacterial immunity may be defined, for our purpose, as a state of resistance of the host, partial or complete, specific or non-specific, towards a given bacterium or its products. The problem is to determine whether allergy, as just defined, plays any part in the resistance of the host to tuberculosis.

Observations on Human Beings.—Stimulated by Scheel's observations at the Ullevaal Communal Hospital at Oslo on the frequency of tuberculosis in probationer nurses, Heimbeck (1927, 1932, 1933, 1936) tested with tuberculin every nurse entering the hospital. The training course lasted three years and comprised a period of service in the tuberculosis wards. Of 905 probationers submitted to the von Pirquet test, 625 gave a positive and 280 a negative reaction. The tests were begun on January 1st, 1924, and up to the time of publication in 1936, 27 of the initially tuberculin-positive and 96 of the initially tuberculin-negative nurses are said to have developed tuberculosis; in the former group none, and in the latter group 10, died. Thus the incidence of tuberculosis was about 8 times as high in the negative as in the positive group.

sensitization has also been shown in experimental animals.³⁰ The significance of heredity in allergy has been said to distinguish this form of hypersensitivity from anaphylaxis but it is not clear that this is a basis of distinction.

Forms of Allergy. The allergic state is manifested in a variety of forms which are determined by two interrelated factors, the portal of entry of the antigen and the tissue predominantly affected, usually referred to as the shock organ. Thus the antigen may be inhaled, ingested, injected or may simply make contact with the skin. The tissues affected are those of the upper respiratory tract, the gastro-intestinal tract and the skin. These combinations give rise to a number of commonly occurring, well-defined clinical types, viz.:

Hay Fever. This is a seasonal allergy produced by pollen grains. Hay fever is a result of hypersensitivity to animal danders, ornitho root (a constituent of many cosmetics) and house dust. The mucous membranes of the upper respiratory tract are affected primarily.

Asthma. Essentially the same inhaled antigens are responsible for asthma as for hay fever and in addition book bindings, straw and similar materials are sometimes involved.

The smaller bronchi are also result from the noted that asthma is a symptom complex and not always allergic in etiology.

Dermatitis. Dermatitis of allergic etiology may result from contact with the antigenic frequently referred to from repeated contact. Ingested antigen not infectious eczema in

periods of time, and is the most common lesion. In angioneurotic edema, or giant urticaria, edema is much more pronounced and the lesions are large pale swellings which cover areas such as the eyelids, lips and genitals. Hives and this edematous kind of lesion are frequently found together and most often result from hypersensitivity to ingested or injected antigen, i.e., foods and drugs.

The foregoing clinical types are characterized by the symptom complex produced, but types of allergy may also be differentiated on the basis of kind and portal of entry of antigen, viz.:

Drugs. In the first instance the symptoms are those of the allergic reaction to the chemical drug, asy. the the

³⁰ Chase Jour. Exp. Med., 1941, 73-711, Jacobs, Kelley and Sommers: Proc. Soc. Exp. Biol. Med., 1941, 48 639.

endemic. On arrival at the mine, they were submitted to a cursory physical examination in order to eliminate those with obvious disease, and were tested intradermally with 0.1 ml. of a 1/5000 dilution of old tuberculin. Their after-history is summarized in Table 101.

TABLE 101

SHOWING PROPORTION OF POSITIVE AND NEGATIVE REACTORS TO 1/5000 TUBERCULIN DEVELOPING CLINICAL TUBERCULOSIS. (S. African gold miners.)

Type of Reaction.	Number.	No. developing Tuberculosis.	Incidence of Tuberculosis per 100,000
(a) Negative	32,864	114	347
(b) Weakly or moderately positive	57,236	391	683
(c) Strongly positive.	3,879	60	1,547
(b and c) Positive	61,115	451	738

It will be seen that the incidence of tuberculosis was more than twice as high in the positive as in the negative reactors, and that the stronger the reaction was to tuberculin, the greater was the liability to clinical tuberculosis.

Before concluding that these figures are in direct contradiction to those of Heimbeck, it is important to realize that the two populations concerned differed in several respects. Heimbeck's nurses had been physically examined before being enrolled as probationers and all those with a history or with symptoms of tuberculosis had been excluded. The South African "boys" had undergone a much less rigorous inspection. As a result, probably a number of the positive reactors were suffering from latent active lesions, which progressed under the severe physical exercise in the mines to manifest pulmonary tuberculosis. This interpretation is borne out by the fact that one-third of the strongly positive reactors broke down within three months of their admission to the mines. It may be noted that, though the incidence of tuberculosis was higher in the positive than in the negative group of Rand labourers, the severity of the disease and the case-fatality rate were both considerably greater among the initially negative reactors. The lesions showed little or no tendency to fibrosis, and the disease became rapidly generalized, whereas in the positive reactors fibrosis occurred and the lesions often remained confined to the lungs. Thus, in 24 per cent. of tuberculous cases in the negative group the disease ran a septicæmic course, as opposed to 4 per cent. in the strongly, and 5 per cent. in the moderately or weakly, positive tuberculin reactors.

Further discrepant observations may be mentioned. Myers and Harrington (1934) in the United States found that children who were tuberculin-positive while at school developed tuberculosis during the next 10 or 12 years far more frequently than their tuberculin-negative classmates. Similar findings were recorded by Pope, Sartwell and Zacks (1939). Yet Zacks and Sartwell (1942) found that in a school for the feeble-minded the proportion of non-reactors who developed tuberculosis during a period of 10 years was 4 times as great as that of initially positive reactors. Levine (1941) found that in children under 6 years of age the fatality rate following exogenous infection was much the same in those who were previously tuberculin-positive as in those who were previously tuberculin-negative, suggesting that sensitization of the tissues did not render a subsequent infection either more or less dangerous. Finally, Badger and Ayzavian (1949) followed up for 5-15 years 745 nurses who took their training at the Boston City Hospital and

if the drug. The drugs commonly

injected. The most common reactions are less frequent.

Food Allergy. The ingested antigens include food as well as drugs and of the latter

sulting disturbance.

Pollen and Dander Allergies. These antigens are inhaled and the symptoms are almost always those of involvement of the upper respiratory tract, most commonly hay fever and bronchial asthma somewhat less so.

Contact Allergies. This is almost entirely the contact dermatitis noted above and is not only an occupational disease but not infrequently results from cosmetics, the lacquers such as nail and hair lacquers, and powders containing orris root.

It will be clear from the foregoing that it is difficult if not impossible to generalize to a satisfactory degree the various interrelated forms of allergy, and the source of the difficulty is that they are not fundamentally different. Some workers divide the clinical allergies into two general groups, atopy (strange disease) or atopic allergy and non-atopic allergy. The atopic allergies include

food and drug allergies make up the non-atopic group. The distinction between the two is quantitative rather than qualitative and it is doubtful whether it is of any real validity, but it does have a certain clinical utility. It was first made on the basis of heredity, hereditary predisposition being an important factor in atopy, and it has even been postulated that in atopy initial contact with the antigen is not necessary for the development of the allergic state. On the other hand, in the non atopic allergies there is almost always a history of contact with the antigen, such as continued exposure to nitrocellulose products in industry, and the hereditary predisposition seems to be of minor importance. There are other correlated characteristics. In atopic allergy the sensitivity is very high, desensitization is difficult and usually only partial at best, skin reactions are marked and specific, and considerable amounts of antibody are demonstrable in the serum. In non atopic allergy the converse generally holds, i.e., sensitivity is low, desensitization is usually successful, skin reactions are weak and non specific, and little antibody is demonstrable in the serum. These distinctions are, however, purely relative and of no fundamental significance.

Allergic Antigen and Antibody. As indicated above, the allergic antigens or allergens (sometimes called atopogens in the atopic allergies) are frequently non protein in nature. This is most obvious in the case of the drug idiosyncrasies in which synthetic substances, such as barbiturates, antipyrine and the like, are clearly not contaminated with protein. Similarly, the substances responsible for contact dermatitis such as lacquers are protein free. The more complex naturally occurring allergens such as pollens, danders and foods are, of course, not protein free but the active agent in some pollens is of lower molecular weight, perhaps 5000, than the usual antigenic proteins. There is

Another difficulty is to know whether the liberal daily dosage with tuberculin necessary to maintain the desensitization of the guinea-pig's skin has any immunizing effect. Rich assumes that it has not. Experimental evidence, it is true, suggests that injection of tuberculin leads to no increase in resistance of the *normal* animal, but that in *allergic* animals it sets up a series of focal reactions which may confer on the tissues some degree of resistance to the invasion of tubercle bacilli.

The work of Lurie (1934) indicates that the destruction of avirulent tubercle bacilli which occurs in the internal organs is greatest when the maximum degree of hypersensitivity is reached. The observations of Freund and Opie (1938) suggest that, though there is no simple relationship between skin sensitivity and resistance to the intravenous injection of virulent tubercle bacilli, nevertheless animals that are moderately allergic at the time of infection usually prove most resistant. Our own observations (Wilson *et al.* 1940) point in the same direction. Thus we found that a moderate degree of allergy in vaccinated animals at the time of infection was more beneficial than a low or high degree, and that in both normal and vaccinated animals the survival time tended to be more or less proportional to the degree of skin sensitivity reached after infection. In this connection the observations of Lurie (1938, 1944) are of special interest. Lurie found that in genetically resistant rabbits the skin was less permeable to Indian ink, and that skin sensitivity to tuberculin was more rapidly established after infection than in genetically susceptible animals. In other words, animals capable of developing the greatest degree of allergy after infection, and presumably of restricting the invasion of the bacilli, tended to have the greatest resistance. The corollary of this, namely that bacilli multiply most in the tissues of animals having a very low degree of skin sensitivity, has been reported by Woodruff and Kelly (1942).

Many years ago Krause (1925) and Willis (1925) showed that the dissemination of tubercle bacilli from a local cutaneous or subcutaneous infection was very much slower in the allergic than in the normal animal. The local fixation of tubercle bacilli may depend, as Rich appears to believe, on the presence of agglutinating antibodies; but since carbon particles (Lurie 1936) and dye particles (Joyner and Sabin 1938) are similarly fixed, it is probable that a non-specific cellular mechanism is at work as well. The observations of Freund and Angervine (1939) indicate that tubercle bacilli may multiply at the site of fixation, and that the retardation of lymphatic invasion is therefore probably due to mechanical factors (see Chapter 47). The mechanism of fixation may be reinforced by the presence of specific antibodies, which tend to accumulate at the site of inflammatory areas in allergic animals (Menkin 1930, Fox 1936).

Intimately bound up with local fixation is the accumulation of mononuclear cells at the site of infection. Lurie (1939*a, b*, 1942) considers the increased phagocytic and destructive power of these cells to be more closely related to immunity than anything else. It is manifested both *in vitro* and *in vivo*, and appears to be independent of humoral antibodies (see also Suter 1953). As a corollary to this there is evidence that the virulence of tubercle bacilli is associated with their rate of growth in these cells (Mackanness, Smith and Wells 1954). The mononuclear cells accumulate more rapidly at the local site of infection in vaccinated or tuberculous animals than in normal animals, and destroy the ingested tubercle bacilli more quickly. It is interesting to note that the suppression of acute inflammation by treatment with tuberculin does not eliminate this increased capacity of the mononuclear phagocytes to destroy tubercle bacilli (Lurie 1945).

All of this evidence is compatible with the thesis that an accelerated response of the tissue cells to the tubercle bacillus or its products is of survival value to the host. This conclusion agrees with that reached by Menkin (1938) in his review on the rôle of inflammation in immunity. The beneficial effect of desensitization with tuberculin seems to us to lie not in abolishing allergy, but in converting a high into a low degree of allergy. The repeated doses of tuberculin set up minor inflammatory reactions around each tuberculous focus in the body, and these tend to retard dissemination of the tubercle bacilli and to prevent the rapid and destructive caseation in the internal organs which seems more

no doubt that haptenic substances can produce the allergic reaction in a sensitized individual, at least in experimental anaphylactic shock, but the question of sensitization is more difficult. High molecular weight non-proteins can act as complete antigens; for example, sensitization may be induced by pneumococcal polysaccharide. It is probable, however, that the relatively simple substances, such as the arsenicals, do not function as sensitizing antigens in themselves, and it is generally believed that they combine with body protein to give a conjugated complete antigen whose specificity is determined by the hapten, and which functions as the sensitizing antigen. It is not clear, however, whether the shocking antigen is such a conjugated one or whether the hapten alone usually produces shock.

It may be noted here that allergic hypersensitivity to physical stimuli, heat, cold and light, may occur. It is unlikely that such stimuli alter body proteins and such allergies probably do not have an

soluble antigen, or its application as a patch on the intact skin, the sensitized individual gives a skin reaction characterized by local erythema and the appearance of a wheal. The skin reaction is more readily elicited and more specific in some allergies than in others, and in general is not satisfactory in contact dermatitis and some of the food and drug allergies. This kind of skin test is to be distinguished from tests like the Schick and Dick tests, in which the reaction is produced by diphtheria or scarlatinal toxin and neutralized by circulating antitoxin, and which have no relation to hypersensitivity.

Antibody is demonstrable in many of the allergies, particularly those grouped under the head of atopy. Its presence may be shown directly in some instances by complement fixation³² but the usual measure is passive transfer of the sensitivity. If a small amount, 0.1 ml. of serum from a sensitized person is

passive transfer cannot be made to guinea pigs, but has been made to rhesus monkeys, and it is probable that a close phylogenetic relationship is essential. This passive transfer was described by Prausnitz and Küstner in 1921 and is known as the Prausnitz-Küstner reaction. A reversed passive sensitization, analogous to reversed passive anaphylaxis, may be produced by inoculation of antigen first, and serum twenty-four hours later.³³ In the usual terminology, the antibody which produces passive sensitization is the allergic reagin, but other antibodies which do not sensitize have also been described. The development of more than one antibody is, of course, to be expected when the antigen contains a mosaic of specificities.

As in the case of anaphylactic shock, the allergic reaction is a consequence of the union of histamine or

³¹ Bronfenbrenner: Jour. Allergy, 1943, 14:105.

³² Hensel and Sheldon Jour. Lab. Clin. Med., 1941, 26 1586.

³³ Wright and Hopkins. Jour. Path. Bact., 1941, 53:243.

institutional and other treatment for suitable patients; the segregation of advanced cases of open tuberculosis; the prevention of overcrowding; the protection of infants and children from contact with tuberculous parents and relatives; the pasteurization or boiling of all milk except that coming from tubercle-free herds; and instruction in personal hygiene and diet (see Discussion 1935, Hart 1937). Special measures are required to protect nurses, medical students, and doctors against the high risk of infection that practice of their profession entails, and to diminish the inhalation of silica dust by industrial workers. Exposure to massive and repeated infection and to unfavourable environmental conditions are particularly to be avoided. A warning should be issued against too rigid an interpretation of Koch's phenomenon, according to which it is often assumed that tuberculous persons cannot be superinfected, regardless of the danger that they incur from allergic reactions around existing foci of disease. (For precautions to be taken in bacteriological laboratories, see Fish and Spendlove 1950)

The argument is sometimes advanced that tuberculous milk is not an unmixed evil. The tubercle bacilli that it contains are presumed to cause mild infections in infants and children which serve to protect them against subsequent infection with the human bacillus. This argument cannot be too strongly condemned. The bovine tubercle bacillus is no less virulent than the human (see Griffith 1925*b*), and many of the infections to which it gives rise are severe and fatal. The argument involves the assumption that the exposed population, as a whole, benefits from the development of an acquired immunity, and ignores altogether the part already played by natural selection in weeding out the least resistant members of the population. Even if it were true, the price to be paid, in terms of lives and suffering, for any such advantage is far too high (see p. 1508 and Griffith 1925*b*, Report 1934). If acquired immunity is to be developed, let us at least endeavour to produce it in some more intelligent way, less fraught with danger of active disease and death.

Our current attitude towards tuberculosis is open to criticism. We spend large sums of money in treating the individual patient, and pay little regard to preventing the disease itself. Until tuberculosis is acknowledged to be an infectious disease and treated as such, it is impossible to expect rapid progress to be made in its control. There is little doubt that if open cases of tuberculosis could be submitted to the same degree of quarantine as were the lepers in the Middle Ages we should eliminate tuberculosis as successfully as leprosy was eliminated from Europe. So long as patients coughing up tubercle bacilli are allowed to travel in public conveyances, to work in offices, factories, and schools, and to live at home with young children, so long will infection continue to be spread and all measures of control be condemned to partial failure. Many students of tuberculosis now recognize this truth. Burnet (1946) in Australia, Frazer (1947) in this country, and Stanko (1946) in Czechoslovakia have pleaded strongly for more radical measures; and the achievements of Myers (1946) and his colleagues in Minneapolis show what can be done in practice. (For the high inverse correlation between the segregation of tuberculous patients and the mortality from tuberculosis, see Stallybrass 1949.)

The aim should be progressively to diminish the total amount of infection as judged by the tuberculin test. The less infective material there is, the fewer the persons who will become infected, and the fewer in turn will be the number of open cases of the disease to distribute infection. A benign circle will be set up and, as in many other diseases, once the proportion of infected to normal persons has fallen below a critical minimum, the disease will probably disappear spon-

mine antagonists such as epinephrine and ephedrine, and a number of synthetic histamine antagonists such as pyribenzamine (N'-pyridyl-N'-benzyl-N-dimethylethylenediamine), benzhydryl, benadryl, antergan and the like have been of considerable interest in recent years and have some therapeutic promise.

Hypersensitivity in Infection. Bacterial cell substance may act as a sensitizing as well as an immunizing agent, and anaphylaxis may be induced with bacterial protein though usually with much greater difficulty than with highly antigenic proteins such as egg albumin and serum proteins. Sensitization may also occur in experimental and naturally acquired infection, but is highly variable, being especially prone to occur with some bacteria and not with others.

Of the bacterial allergies by far the most thoroughly studied is that developed to the tubercle bacillus. It is demonstrable as a delayed (one to four days) local inflammatory skin reaction, the tuberculin reaction, to preparations of soluble antigen of the tubercle bacillus known as tuberculin. The tuberculin reaction is considered in detail elsewhere (p. 638). This kind of reaction is somewhat different from the immediate wheal type of reaction, with respect to time of development, the nature of the dermal reaction, and in that it is not passively transferable. The generalized response also differs from the immediate shock of anaphylaxis, the inoculation of a tuberculous guinea pig with tuberculin in a dose sufficient to kill does not result in death until after some hours, and on autopsy the site of inoculation is congested, focal glands are swollen and congested, and focal reactions occur about tuberculous lesions which consist of areas of enormous dilatation of the capillaries. This is obviously different from fatal anaphylactic shock in the same animal. The nature of the antigenic stimulus inducing tuberculin-like sensitivity has been shown by Raffel¹⁴ to be a protein combined with a wax fraction of the bacilli, the protein alone giving the usual immune response with the formation of precipitins.

Hypersensitivity is the outstanding immunological response to infection with a number of other microorganisms, and the skin reactions have been of some interest from a diagnostic point of view. In brucellosis, for example, a marked degree of hypersensitivity is developed and a skin reaction, a slightly raised edematous area, appears in about six hours after the intradermal inoculation of preparations of soluble antigen of *Brucella*. The preparations have been given various names such as abortin (from *Br. abortus*), melitin (from *Br. melitensis*), brucellin and brucellerger. Johnin, a preparation of John's bacillus, *Mycobacterium paratuberculosis*, is used in the diagnosis of John's

as a skin reaction following intradermal inoculation of killed Ducrey's bacillus, and a hypersensitivity occurs in leprosy which results in a positive skin reaction to extracts of leprosy tissue termed lepromin. Hypersensitivity to fungi is also not uncommon; the mycids (p. 698) or secondary sterile lesions which occur

¹⁴ Raffel, *Jour. Inf. Dis.*, 1948, 82 267.

(Birkhaug 1944, Rosenthal *et al.* 1948) or by scarification (Nègre and Bretey 1947) is preferred by some workers. Segregation of infants of tuberculous families is recommended both before and after vaccination; and the vaccine is widely used, not only for infants, but for tuberculin-negative children, adolescents, and young adults.

Though B.C.G. vaccination has been used extensively in different countries of the world, its value is extremely hard to assess. The main difficulty is that, with a few exceptions to be mentioned later, most observers have failed to realize the necessity of comparing the vaccinated subjects with a properly selected control group as nearly identical as possible in age, sex, colour, social and economic status, and general environment as the vaccinated group, and treated and followed up in exactly the same way over the same period of time (see Wilson 1947). The usual practice has been to compare the tuberculosis or general mortality in the vaccinated infants with the estimated mortality for non-vaccinated infants in similar surroundings or in the country as a whole. Such comparisons, as Greenwood (1928), Rosenfeld (1928), Wolff (1930*a, b*), and Berghaus (1930, 1931) have pointed out, are grossly unfair. In the first place no exact figures exist for the mortality in unvaccinated infants brought up in a tuberculous environment, and the estimates that have been made differ enormously. In the second place, the unvaccinated infants are not strictly comparable with the vaccinated group, partly because they are not kept sheltered from infection during the first 2 months of life, and partly because they receive less parental, medical, and nursing attention than those infants which, after For these reasons, f data

that has accumulated on this subject in France and French-speaking countries would serve no useful purpose, since from a statistical point of view it is practically worthless.

The realization of these fallacies has now led to the institution by other workers of a few experiments in which an attempt has been made to provide a control group with which the vaccinated group can be more strictly compared. It must be noted that the difficulties of obtaining an absolutely identical control group are almost insuperable, and some allowance has therefore got to be made when considering the results. We have already (p. 1523) drawn attention to the favourable results recorded by Heimbeck following the vaccination of von Pirquet-negative probationer nurses with B.C.G. Similar results were also obtained by Nordwall (1944) in Stockholm and Ferguson (1946) in Canada with nurses and by Scheel (1935) with medical students. We may now briefly consider the results recorded by other workers on the vaccination of infants.

The early controlled investigations in the United States of Park, Kereszturi, and Mishulow (1933) and of Aronson and Dannenberg (1935) suffered from various defects, and not too much attention can therefore be paid to the mildly favourable results obtained in the vaccinated infants.

Rosenthal and his colleagues (1945, 1948) carried out a careful series of observations on infants in Chicago.

(a) In one series alternate infants born at the Cook County Hospital were vaccinated by the multiple puncture method during the first few days of life, and were followed up along with the control unvaccinated infants. The infants lived in a highly infected part of Chicago, but were not exposed to contact with tuberculous in the household. The investigation was continued over a period of ten years. The results were as follows:

temperature. Considerable resistance is displayed toward drying, experiments showing a retention of vitality for many days and even months in cultures dried upon silk threads and desiccated over calcium chloride. Toward the chemical substances ordinarily used as disinfectants the staphylococci also exhibit more than the average resistance. They are, in general, among the hardiest of the non-spore-forming bacteria.

Toxins.¹⁰ A variety of toxic substances are produced by staphylococci, including hemolysins, leucocidin, coagulase, fibrinolysin, spreading factor (Duran-Reynals factor), skin-necrotizing substance, a lethal factor and enterotoxin.

Hemolysins. The pyogenic staphylococci are almost invariably β -hemolytic on blood plates and produce filterable hemolysins in broth culture, while the saprophytic forms are less frequently hemolytic. The filterable hemolysins



Fig. 43. Colonies of *Staphylococcus aureus* on nutrient agar. Twenty-four-hour culture, $\times 3$.

(staphylolynsins) are of two types, one lysing red cells upon incubation (α -lysin) and the other (β -lysin) only after holding in the icebox following preliminary incubation—the so-called “hot-cold” lysis (p. 207).

Other Toxins. The ability of many pyogenic staphylococci to coagulate citrated plasma, lyse fibrin clots, kill leucocytes and increase the permeability of the skin may be shown by appropriate techniques. Sterile filtrates from broth cultures produce necrosis upon intradermal injection—the so-called skin-necrotizing factor—and almost immediate death when injected intravenously into rabbits. Whether these and other effects are due to the activity of separate substances or are various manifestations of the activity of a single substance is uncertain. It is definitely established that more than one hemolysin is produced and that the enterotoxic substance, effective *per os* and of considerable food-poisoning significance (p. 272), is not identical with the other activities. More than one toxic substance is produced, then, but probably some of these produce more than one effect. In general, toxin production is a property of the pathogenic staphylococci, usually of the *aureus* variety

¹⁰ Cf. Blair: Bact. Rev., 1937, 3:97.

the X-ray films did not know to which group any given subject belonged. The study lasted for seven years. The results are given in Table 103.

TABLE 103

B.C.G. VACCINATION OF NORTH AMERICAN INDIAN CHILDREN AND YOUNG ADULTS
(Aronson and Palmer 1946)

Group	No. of Subjects	No. Developing N.P.T.	No Developing P.T.	Tb Case Rate per 1,000 Person-years ¹	No of Deaths from all Causes	No of Deaths from Tb
Vaccinated . . .	1,550	3	14	2.0	34	4
Non-vaccinated . .	1,457	19	49	9.0	60	28

¹ Cases of pleural effusion and enlarged hilar glands excluded.

N.P.T. = Non-pulmonary tuberculosis. P.T. = pulmonary tuberculosis.

It will be noted that the incidence rate of tuberculosis was 4-5 times as high in the control as in the vaccinated group, and the number of deaths from tuberculosis 7 times as high.

Against these favourable results must be quoted the experience of Blanch, Blanch, and Lieutier (1945) in Uruguay. About 50,000 new-born infants living in a highly infectious environment were vaccinated with B.C.G. Of 128 vaccinated children that were followed up 38 per cent., and of 141 controls 38 per cent., developed tuberculosis as judged by clinical and radiographical examination. The mortality among the vaccinated was 7 per cent. and among the controls 5.6 per cent. The authors conclude that B.C.G. vaccination is powerless to protect against heavy infection with tubercle bacilli.

So far as deductions from such small numbers will allow, these results confirm those of Levine and Sackett in New York, and point to the necessity of segregating infants for two months or so after vaccination if they are liable to be exposed to infection.

Hyge (1947) describes a small outbreak of tuberculosis that occurred in a girls' school in Denmark, apparently caused by a tuberculous teacher. Nine months previously the children had been tuberculin-tested, and rather over half of the negative reactors had been vaccinated with B.C.G. Of 93 unvaccinated children, 40 developed lesions of pulmonary tuberculosis as judged by X-ray examination or the presence of tubercle bacilli in stomach washings or both, and 6 required artificial pneumothorax treatment: of 107 vaccinated children, 3 developed pulmonary lesions and 2 required treatment by artificial pneumothorax. These differences are striking. There are, however, a number of anomalies in the account of this outbreak which suggest caution in accepting the results at their face value.

Hyge's findings are very different from those quoted by Bergqvist (1948), who observed the development of 18 cases of tuberculosis among 115 students in the Stockholm Dental School who were in contact with an open case of the disease. The distribution was as follows: 4 out of 57 (7 per cent.) of tuberculin-positive unvaccinated, 10 out of 44 (23 per cent.) of tuberculin-positive vaccinated, and 4 out of 14 (29 per cent.) of tuberculin-negative persons. Here it is evident that vaccination afforded little or no protection.

Two more experiences may be referred to, though we have no space to describe them in detail. Hertzberg (1948), working in the Tuberculosis Clinic at Oslo, compared the incidence of tuberculosis, not between a vaccinated and a control group, but between B.C.G. vaccinated persons who became infected—as judged by an increase in the intensity of their tuberculin reaction—and a group of unvaccinated tuberculin-negative reactors who became tuberculin-positive. Vaccination appeared to confer considerable

Variation. Like other bacteria, the staphylococci dissociate, and both rough and G colonial types have been described.¹¹ These have not been thoroughly studied, however, and are not well known.

Classification. The genus *Staphylococcus* was formerly one of four which made up the family *Micrococcaceae* but the generic name has been dropped by Bergey (1948) in favor of *Micrococcus*. The staphylococci are roughly divisible into two types, the one designated as aerobic, though the bacteria are in fact facultative anaerobes, and the other which is strictly anaerobic. The common species comprising the former group, which is by far the better known, are the pigmented forms *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*), which is golden yellow and generally pathogenic, and *Staphylococcus citreus* (*Micrococcus citreus*), which is lemon yellow and a saprophyte, together with the non-pigmented *Staphylococcus albus* (*Micrococcus pyogenes*

Staphylococcus aerogenes, *Staphylococcus asaccharolyticus*, *Staphylococcus anaerobius*, *Staphylococcus niger* and *Staphylococcus grigoroffi*. These anaerobic species are inhabitants of the body cavities, and the first is occasionally the cause of puerperal fever

Physiological Differentiation. The validity of these species is, however, open to serious question. The differentiation of the better known aerobic species on the basis of pigment production is of doubtful value, for this characteristic is variable. Recently isolated pyogenic cocci forming a rich golden pigment lose this property on continued cultivation on artificial media and become identical with the *albus* variety. No biochemical tests serve to sharply differentiate these bacteria from one another. The strains that produce white colonies are, as a rule, less active in gelatin liquefaction and fermentative power, and hence have been sometimes regarded as weakened relatives of the biochemically more vigorous golden pigmented types. The *albus* strains are also as a rule only feebly pathogenic. Dextrose, maltose and glycerol are fermented by nearly all *aureus* strains and by a consistently lower proportion of *albus* strains, lactose and mannitol by about four fifths of the *aureus* and by about one third (mannitol) to two-thirds (lactose) of the *albus* strains.¹² In general, the types most commonly isolated from air, dust and other sources outside of the human body are white staphylococci, while those found associated with pathologic conditions are *aureus* strains. The general opinion is that the staphylococci form a closely graded series from the pigmented, hemolytic, gelatin liquefying, pathogenic, actively fermenting strains to those that are unpigmented, feebly pathogenic and less actively hemolytic, liquefying and fermenting.

Immunological Differentiation. Differentiation of the staphylococci on the basis of agglutination reactions has as yet been unsatisfactory. It has been shown,¹³ however, that two immunological types may be differentiated by pre-

¹¹ Hoffstadt and Youmans. Jour. Inf. Dis., 1932, 51: 216. Bigger, Boland and O'Meara. Jour. Path. Bact., 1927, 30: 261. Swingle. Jour. Bact., 1935, 29: 467.

¹² Dodgeon and Simpson. Jour. Hyg., 1927, 27: 160.

¹³ Cf. Jubanille and Wiegand. Jour. Exp. Med., 1935, 62: 11, 23, 31.

Griffith 1934-35), and sometimes to generalized lesions (van der Hoeven 1941a); it has been isolated from the sputum, milk, intestine, and mesenteric glands (Wolters 1931, Glover and Griffith 1934-35, Glover 1941). Pigs suffer from generalized and from localized infection with the bovine, and from localized infection with the avian and much less often the human type. Nearly all strains isolated from the horse have been of bovine type; but they are often atypical and of less than standard virulence for calves and rabbits (Griffith 1937b). In the dog the human type seems to be commoner than the bovine (see Lovell and White 1940); but all strains from the cat have proved so far to be of bovine type. Sheep may be infected with the bovine or avian type; in the United States Harshfield, Roderick and Hawn (1937) found the avian type in 25 out of 26 cases. Parrots kept as pets

TABLE 104

TYPES OF TUBERCLE BACILLI FOUND IN CERTAIN DOMESTICATED AND CAPTIVE WILD ANIMALS
(Mainly English Investigations.)

Species of Animal.	No. of Cases	Type of Tubercle Bacillus		
		Bovine.	Human.	Avian
Anthropoid Apes	6	—	6 ¹	—
Lemurs	2	—	2	—
Monkeys	26	8	18 ²	—
Cattle	52	50	1	1
Horse	43	42 ³	—	1
Sheep	17	14	1	2
Goat	13	13	—	—
Deer	3	3	—	—
Gnu	3	2	1	—
Antelope	3	3	—	—
Pig	258	183 ⁴	5 ⁴	70 ⁵
Cat	34	33 ⁷	1	—
Dog	116	34	82	—
Rabbit	8	4	—	4
Guinea-pig	6	5	1	—
Ferret	1	1	—	—
Parrot	1	—	1	—
Fowls, and other birds	26	—	1	25

¹ Including 1 strain of sub-standard virulence.

² Including 1 dysgonic strain.

³ Including 17 strains of sub-standard virulence.

⁴ Including 4 cases in which avian, and 1 case in which human, bacilli were also present.

⁵ Including 1 case in which bovine bacilli were also present.

⁶ Including 4 cases in which bovine bacilli were also present.

⁷ Including 1 strain of sub-standard virulence.

are usually infected with the human type, but those living in aviaries with other birds may be infected with the avian type (see Cobbett 1917). Fowls and most other birds are infected almost exclusively with the avian type of bacillus.

Tuberculosis of Cattle.—Tuberculosis is one of the most serious diseases of cattle. In 1946 it was estimated that 17-18 per cent. of all cattle and 30-35 per cent. of all cows in Great Britain reacted to the tuberculin test (Ritchie 1945-6). About 40 per cent. of animals slaughtered in public abattoirs show macroscopic lesions of tuberculosis, and about 0.5 per cent. of all milch cows excrete tubercle bacilli in their milk (Report 1932a, 1934, Savage 1933). The disease is common throughout

cipitation tests with specific carbohydrates extracted from the microorganisms. One of these types, designated type A, is composed for the most part of strains isolated from pathogenic sources, while the other, type B, comprises strains of non-pathogenic origin. The majority of type A strains ferment mannitol, while those of type B do not.

The Coagulase Test. The ability of staphylococcus strains to coagulate plasma appears to be associated with pathogenicity in that the majority of strains isolated from pathologic processes are coagulase-positive while the saprophytic strains are usually coagulase-negative. It has been suggested that the staphylococci be divided into pathogenic and non-pathogenic types on this basis, and in recent years the coagulase test has been generally used.¹⁴ It has been found also that coagulase-positive strains clump rapidly when mixed with fresh human plasma, the CO₂-soluble fraction of fibrinogen seems to be the most important part of the plasma in effecting immediate clumping.¹⁵ The slide test¹⁶ for clumping, carried out by mixing fresh, undiluted human plasma with heavy bacterial suspension, is a simple and rapid means of differentiation.

Pathogenicity for Man.¹⁰ Experimental evidence and that of comparative pathology show that man is more susceptible than the ordinary laboratory animals to staphylococcus infection. Garre¹⁷ inoculated himself by rubbing a pure culture upon the uninjured skin of the forearm, with the result that a series of carbuncles was produced, seventeen scars remaining to testify to the success of the experiment. The penetration of the cocci into the deeper layers of the intact skin, probably through the sweat ducts or at the base of the hair follicles, is a fact of considerable significance. The positive occurrence of such penetration seems well established, and the negative observations of some authors may well be referred to differences in the virulence of the strains employed or to other experimental discrepancies.

The demonstration that staphylococci have power under certain circumstances to penetrate the skin, taken together with their practically constant presence upon the skin itself, serves to explain the multiplicity of human affections with which these microorganisms are found associated. A momentary weakness on the part of the tissues in almost any locality may lead to a rapid local invasion, followed by the production of a simple boil or by a more or less extensive carbuncular condition. Septicemia and pyemia sometimes result through the introduction of staphylococci into the lymphatics or the blood stream from a local abscess. The initial lesion may be trivial in character. In the series of 122 cases studied by Skinner and Keefer¹⁸ the case fatality rate was 82 per cent. Rapidly fatal bacteremia may occur without metastatic abscesses or metastatic abscesses may be produced, or the bacteremia may clear but leave focal abscesses and the infection still be fatal.

Staphylococci are not only found frequently in all parts of the body in secondary and mixed infections, but they are also primarily responsible for a variety of specific pathologic conditions and for injury to particular organs.

¹⁴ For details see Fisk. Brit. Jour. Exp. Path., 1940, 21:311, Gillespie. Med. Res. Council (Great Britain) Monthly Bull., Emergency Pub. Health Lab. Service, 1943, 2:19.

¹⁵ Berger. Jour. Path. Bact., 1943, 55:435.

¹⁶ Cf. Cadness Graves, Williams, Harper and Miles. Lancet, 1943, 1:736.

¹⁷ Garre. Fortschr. d. Med., 1885, 3:165.

¹⁸ Skinner and Keefer. Arch. Int. Med., 1941, 68:851.

The most satisfactory method is to draw off cream

It has been shown that tubercle bacilli in milk are generally associated with the presence of characteristic endothelial cell clumps. It is therefore only necessary to examine the film with a low power for the presence of these clumps, and to reserve the oil immersion lens for films in which these clumps are present. The clumps themselves are not diagnostic of tuberculosis, but they afford a good indication of the locality of the tubercle bacilli in the film. It would appear that about 50

Inoculation is not often used as a routine, since it is less delicate than the guinea-pig method and not as rapid as the microscopic method. It is suitable mainly for the milk of individual cows collected under aseptic conditions.

The sediment resulting from high-speed centrifugation should be treated with an equal volume of 15 per cent. HCl for 10-15 minutes, and inoculated on to suitable media (see Wolters and Dehmelt 1931).

The main value of the method seems to be in the occasional detection of the avian tubercle bacillus, which might be missed by the animal inoculation method.

Scrological methods are still in the experimental stage. German workers have reported favourably on the value of the complement-fixation test carried out on the milk whey in the diagnosis of udder tuberculosis (see Karsten 1933, 1935, Sontgen and Menck 1933, Rautmann 1934, Rautmann and Hartwig 1934, Schultz 1935, Paarman 1936, Kuhlmann 1936), but there is little evidence to suggest that they furnish a reliable index of the infection of the milk.

Guinea-pig inoculation constitutes the most generally useful and most delicate method of detecting tubercle bacilli in milk. Fresh milk should be used, since other organisms, such as certain streptococci, may produce substances during growth having a germicidal action on the tubercle bacillus (Mattick and Hirsch 1946).

About 50-100 ml. of milk, either mixed or from individual animals, should be centrifuged at 3,000 r.p.m. for 30 minutes, after removal of the gravity cream. The sediment and the cream should be mixed, and 3 ml. of the mixture should be injected intramuscularly into the left thigh of each of two animals, which should previously have been shown to be free from tuberculosis by means of the intradermal tuberculin test. One animal should be killed 3-4 weeks after inoculation; if the results are negative, the other animal should be left for 6-8 weeks. The post-mortem appearances of animals inoculated with tubercle bacilli have already been referred to (p. 512), and it need only be mentioned here that no animal should be regarded as tuberculous unless characteristic lesions containing acid-fast bacilli are demonstrated.

The Tuberculin Test in the Diagnosis of Cattle Tuberculosis.—This test may be carried out in a number of different ways, but each method consists in noting a reaction, local or constitutional, to the injection of tuberculin. (a) *Intradermal test.* This consists in injecting tuberculin, or more commonly the Purified Protein Derivative (P.P.D.), into the skin of the neck (Great Britain) or the caudal fold (U.S.A.) and noting the size and nature of the local reaction 3-5 days later. In this country the double intradermal test (Report 1925, Buxton and MacNalty 1928) was generally used up to 1917, but it has now been replaced by the single injection method. (b) *Ophthalmic test.* Tuberculin is dropped into the conjunctival

Many lesions and diseases of the skin have been attributed to staphylococci. In the case of some of these it has been claimed that special varieties or races are concerned, but the characters said to distinguish these from the ordinary *Staphylococcus aureus* or *albus* are not, as a rule, of differential value.

A considerable majority of all attacks of acute osteomyelitis and periostitis are due to staphylococci, which appear to have a special predilection for the tissues of the osseous system.

Suppurative inflammation, in whatever part of the body it may occur, is usually associated with the presence of staphylococci either in pure or mixed cultures. Sometimes when found in a mixed infection they are doubtless the original exciting cause, in other cases they may have arrived at the seat of trouble only after a primary invasion by some other bacterium. In a given instance it may be impossible to determine the precise sequence of events.

Staphylococcus infection of the lung sometimes occurs, and the resulting bronchopneumonia is often fatal. Out of about 800 patients with pneumonia treated at the Hospital of the Rockefeller Institute in New York City from 1913 to 1918, 13 were infected with staphylococci, and 10 of the 13 died. Under certain conditions, as during the 1918 influenza epidemic at Camp Jackson, *Staphylococcus aureus* may play an important part in the pneumonia complicating primary infections. Chickering and Park¹⁹ found that in 49 per cent of 312 postmortem lung cultures this microorganism was present either alone (92 cases) or in association with other bacteria. Similarly, Gaspar²⁰ found that of 144 fatal cases of pneumonia cultured at autopsy, 38 were caused by staphylococci. In this series about two-thirds of the staphylococcus pneumonias occurred in the first decade of life. The presence of staphylococcus in the lungs is usually interpreted as a secondary invasion in the train of some primary exciting agent.

Staphylococcus food poisoning has been discussed in a previous chapter (Chap. 11).

Bacteriological Diagnosis. The isolation of staphylococci from pathologic material or from foods suspected in outbreaks of food poisoning is a simple matter. Blood agar is the medium of choice since the pathogenic strains are usually hemolytic *Staphylococcus aureus*, and the hemolytic character is apparent on this medium. Examination of gram-stained smears from golden yellow hemolytic colonies will show the characteristic morphology. Colonies may be picked for the coagulase test (see above) but fermentations are not significant and immunological typing is desirable only in special studies. The ability to form enterotoxin in the case of food poisoning strains may be demonstrated by feeding sterile filtrate of subcultures incubated in 25 per cent CO₂ to human volunteers in amounts of 2 to 5 ml., or to rhesus monkeys by stomach tube in 25 to 50 ml. amounts. The kitten test for enterotoxin is not reliable.

Pathogenicity for Lower Animals. The rabbit has proved one of the more favorable animals for experimentation, intravenous injection of broth cultures being the most successful mode of infection. A moderately virulent strain kills an average sized rabbit in four to eight days after injection of 0.1 ml.

¹⁹ Chickering and Park. Jour. Amer. Med. Assn., 1919, 72:617.

²⁰ Gaspar. New York State Jour. Med., 1941, 41:834.

has been adopted, aiming at the elimination not only of open cases of tuberculosis, but of all animals infected with the tubercle bacillus. The general method is to test every animal in the herd with tuberculin, to segregate, remove, or slaughter the positive reactors, and to build up the herd exclusively from negatively reacting animals. The tuberculin test must be repeated at 3- or 6-monthly intervals to detect any fresh reactors in the clean herd. Once a sufficiently high proportion of herds in a given area have been freed or almost freed from infection, the area can be scheduled as an accredited or attested area, and the remaining herds are then compulsorily brought into the eradication scheme. This method, if accompanied by complete removal or slaughter of the reacting animals, is often highly successful in eliminating the disease. Clean herds must, however, be tuberculin-tested at intervals to see that infection is not re-introduced. In the United States of America the proportion of animals reacting to tuberculin was reduced between 1917 and 1940 from an average initial rate of about 4 per cent. to one of about 0.5 per cent. (Myers 1940) and to 0.18 per cent. by 1943 (Report 1946). Scandinavia has also achieved great success by the use of this method; and the introduction of the Attested Herds scheme into Great Britain in 1935 had resulted by 30 September 1953 in the registration of 43.5 per cent. of the cattle as attested.

Vaccination of Cattle against Tuberculosis.—Various vaccines have been used in an endeavour to control the disease. Von Behring vaccinated calves with living bacilli of the human type, but though the method had some success it had to be discarded when it was found that the bacilli remained alive in the tissues and were excreted in the milk (Report 1911c, Griffith, A. S., 1912-13, 1916-17). Avian bacilli were tried, but with less success, probably because they do not set up the same focal lesions in cattle that human bacilli do (McFadyean *et al.* 1913, Gamma and Giordano 1926). Friedmann introduced his turtle bacillus for vaccination, but this organism was found to provide little or no protection for warm-blooded animals. Calmette and Guérin's B.C.G. vaccine (see p. 1529) holds out greater promise. It has been tested by several workers in different countries (see Watson 1928, 1930, Schroeder and Crawford 1928-29, Rankin 1929, Rankin *et al.* 1932, Haring *et al.* 1930, Bang, O. *et al.* 1931, Cotton and Crawford 1932, Jundell and Magnusson 1933, Griffith, Buxton, and Glover 1931, 1935, Buxton, Glover and Griffith 1934-35, Ascoli 1936, Buxton 1936, Buxton *et al.* 1939, Report 1937, Dalling 1946, Glover and Ritchie 1953). There seems to be little doubt that, if B.C.G. is given to uninfected calves shortly after birth, preferably by the parenteral route, their resistance both to experimental and natural infection with the tubercle bacillus is definitely increased compared with that of unvaccinated control animals. The degree of immunity produced is limited and seems to wear off in the course of a year or two unless re-vaccination is practised. How much help B.C.G. vaccination will afford in practice in the control of tuberculosis is doubtful. In heavily infected herds vaccination of calves might possibly serve to protect them while the reacting stock was being gradually removed from the herd, thus paving the way for a proper eradication scheme. But the most heavily infected herds in this country are "flying herds," which do not retain their calves. In heavily infected breeding herds the calves may be infected from tuberculous milk soon after birth, thus rendering vaccination inapplicable. Less heavily infected herds can be freed from infection more easily by immediate removal, or by removal after isolation, of tuberculin reactors found at the first test. B.C.G. vaccination renders the animal sensitive to tuberculin, and thus interferes with the eradication scheme. The

of a twenty-four-hour broth culture. On autopsy, minute abscesses are found in various internal organs, most commonly in the kidney (particularly in the cortex) and in the walls of the heart. Under ordinary conditions of experiment with healthy adult rabbits the bone marrow and periosteum are rarely seriously affected. In young animals, however, several workers have claimed to have evoked typical osteomyelitis by intravenous injection of staphylococcus cultures. It is perhaps questionable whether in these cases the processes in the affected tissues are strictly comparable with natural osteomyelitis in man. The injection of cultures into a rabbit suffering from a fractured bone or an injured periosteum produces a more characteristic chain of events, and one that closely resembles the course of human osteomyelitis. Rabbits are relatively insusceptible to intraperitoneal inoculation with staphylococci. Artificial inoculation



Fig. 44. *Micrococcus tetragenus*; smear from pure culture stained with fuchsin. Note the relatively large size of the cells and the typical tetrad arrangement with the irregular clumps tending to be made up of tetrads. $\times 1800$.

of the eye, on the other hand, succeeds readily, although natural eye infection is never observed. Feeding experiments with staphylococci do not produce infection. White mice are sometimes used for inoculation experiments, but are less uniformly responsive than rabbits; guinea pigs are relatively resistant, rats and pigeons highly so.

Cases of spontaneous staphylococcus infection among domestic animals, while not so common as in man, are not unknown. In horses and cattle *Staphylococcus aureus* has been found associated with pathologic processes and conditions similar to those in man. Mastitis in cattle is not uncommon. In sheep, "staphylococcus mastitis" is a well-known condition. In birds, "staphylococcus mastitis" is also known. In horses, "staphylococcus mastitis" is also known. In cattle, "staphylococcus mastitis" is also known. In sheep, "staphylococcus mastitis" is also known. In birds, "staphylococcus mastitis" is also known.

special species of staphylococcus, "Staphylococcus bovis" (in cattle) and "Staphylococcus hemorrhagicus" (in sheep), but such species are of doubtful validity. Typical strains of *Staphylococcus aureus* and *albus* have been isolated from spontaneous abscesses in birds.

²¹ Cf. Minett Jour. Comp. Path. Therap., 1937, 50-101; Plastringe, Anderson, Williams and Weirether. Storrs Agr. Exp. Station Bull. No. 231, 1939.

3 per cent. of market eggs, and 10 per cent. of eggs from tuberculous hens, contained tubercle bacilli. In the United States a survey of 115,700 flocks of poultry in 40 States revealed the presence of tuberculosis in 6,690 (5.8 per cent.); the actual number of fowls affected was estimated at over 8 million (Mohler and Washburn 1930). The highest incidence of infection occurs in the north central states. Disease is much commoner in old birds than in young (Feldman 1938). The organs most frequently affected are the liver, spleen, intestines, lungs, bones, joints, peritoneum, kidneys, and ovary (Gallagher 1926). The disease can be diagnosed by the intradermal tuberculin test using avian tuberculin. The slaughter policy seems to be the most suitable method of dealing with the disease when it is extensive, but an eradication plan based on the use of tuberculin may be tried if the disease is less extensive or the birds valuable. By this means the proportion of flocks in the United States of America reacting to tuberculin was reduced from 6.2 per cent. in 1925 to 3.0 per cent. in 1945 (see Report 1946). (For a general survey of avian tuberculosis see Gloyne 1933, Feldman 1938.) Acid-fast organisms have been reported in bumble-foot of chickens, but their exact nature is unknown (see Bunyca 1936).

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Immunity. An active immunity may be developed against staphylococcal infection by immunization with vaccines. In the rabbit the use of killed suspensions followed by living attenuated bacteria occasionally results in infection, and the inoculation of living microorganisms is somewhat dangerous. Immunization of human beings is, therefore, confined to the use of killed suspensions. The immunity so induced is in part antibacterial and in part antitoxic if the toxic products of the microorganism are included in the vaccine. The antibacterial immunity appears to be essentially a stimulation of the phagocytic mechanisms; the rate of phagocytosis is remarkably increased, and the activity of the phagocytic cells is of prime importance in combating infection with these bacteria. According to Valentine and Butler²² antileucocidin is of considerable importance in the immunity. Antibacterial sera will protect untreated animals against infection, and the acquired immunity is associated with an increase in the amount of opsonin.



Fig. 45. Colonies of *Micrococcus tetragenus* on blood agar. Twenty four hour culture. $\times 3$.

The therapeutic use of vaccines in the treatment of boils and carbuncles and chronic furunculosis in man has met with some success. There is good evidence that the autogenous staphylococcus vaccine (the strain cultivated from the patient) is more efficacious than the ordinary stock vaccine.

Considerable interest has attached to the antitoxic aspect of antistaphylococcus immunity. Toxoid, prepared by treating staphylococcus culture filtrates with formalin, has been used in producing an active antitoxic immunity, and a number of attempts have been made to demonstrate the efficacy of antitoxic sera. The therapeutic use of toxoid has given encouraging results in some instances, and it is of interest that the antileucocidin appears to be of particular significance.²³ The value of antitoxic sera is uncertain. Their use, either through parenteral inoculation or local application, has not led to unequivocal results though in a number of instances very favorable results have been re-

²² Valentine and Butler. *Lancet*, 1939, ii 973.

²³ Cf. Haum. *Acta Path. Microbiol. Scand.*, Suppl. 35, 1938.

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ported.²⁴ The antitoxin, it may be noted, is standardized in terms of anti-hemolysin, one unit being that amount which will neutralize 200 minimum hemolytic doses of toxin; in consequence the toxin is sometimes differentiated into α -toxin and β -toxin, referring to the α - and β -lysins.

OTHER MICROCOCCI

A variety of micrococci, both pigmented and otherwise, have been described, most of which are saprophytic forms found in water and elsewhere in nature. A well-known representative of this group is *Sarcina lutea*, a coccal form producing a bright yellow pigment which derives its generic name from a tendency to form cubical packets of eight cells.

Micrococcus tetragenus (*Gaffkya tetragena*) is a parasitic coccus frequently found on the mucous membranes of the upper respiratory tract. It was discovered by Gaffky²⁵ in the pulmonary cavities in phthisis, and has been found in pure culture in abscesses in animals and man, and often occurs in the healthy mouth. Morphologically *Micrococcus tetragenus* is distinguished by its occurrence in tetrads or groups consisting of four small oval cocci. It is gram-positive. In cultures the sheet-like arrangement is not always seen, but in the animal organism the flat tablets occur uniformly, and a rather heavy capsule surrounds the tetrad. On agar a confluent rough, elevated white growth is produced. On potato a thick, white, slimy growth occurs. Gelatin is not liquefied, milk is coagulated. Growth is slow and occurs at 20° and at 37° C., though better at the higher temperature.

White mice inoculated with *Micrococcus tetragenus* succumb to a rapidly progressing septicemia. Guinea pigs and rabbits usually show only a local affection. House mice and rats are relatively resistant. Fornaca²⁶ has reported a case of septicemia in man in which *Micrococcus tetragenus* was present in pure culture in the blood. It is not uncommonly found in suppurations of the mouth and neck. It is also found in the empyema following pneumonia and in the pus of war wounds.

This microorganism is probably of low-grade virulence, and unable, as a rule, to invade the human tissues except when the resistance is lowered by some depressing influence, especially of the kind caused by the invasion of some other bacterium.

²⁴ Cf Kleiger and Blair Arch. Surg., 1943, 46:548.

²⁵ Gaffky: Mitt. a. d. k. Gesund., Berlin, 1881, 1.1.

²⁶ Fornaca: Rif. Med., 1903, 19:309.

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THE STREPTOCOCCI

The streptococci make up a relatively large group of pyogenic coccus forms characterized by an arrangement of the cells in chains. They are responsible for a variety of diseases of man, certain diseases of lower animals, and some are saprophytes found in milk and milk products. They were early observed in the pus formed in suppurative inflammatory conditions, and their frequent presence and pathologic significance were first emphasized by Ogston, by Fehleisen and by Rosenbach in the early 1880's. It is now well known that, in addition to the more virulent pathogenic forms, relatively harmless parasitic streptococci are more or less constantly present in the human throat and in

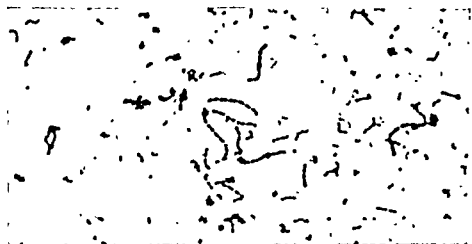


Fig. 46. *Streptococcus pyogenes*. Recently isolated scarlet fever strain. Note the tendency to diplococcus arrangement in the chains. Fuchsin, $\times 1050$.

the intestinal tract which assume a pathogenic role only under circumstances in which normal resistance is markedly reduced, and which may be regarded, for all practical purposes, as a portion of the normal flora of the human body.

Morphology and Staining. Like staphylococci, individual streptococci are spherical and 0.8 to 1.0μ in diameter. Some variation in size results from the character of the culture medium, and the individual cells are frequently appreciably smaller when the cultures are grown under anaerobic conditions. Smaller varieties, 0.4 to 0.8μ in diameter, whose size is apparently a constant character, have been described. The typical streptococcus divides in only one plane, and the tendency of the cells to remain united results in the development of the characteristic chains that give these organisms their generic name.

This tendency is apparently more pronounced between daughter cells following the first cell division, and the chains frequently have the appearance of chains of diplococci, with pairs closer to one another than to adjacent pairs; this is apparent in Fig. 46. The firmness of the attachment is to some degree a strain characteristic and some strains appear as relatively long chains while others show little more than two pairs of diplococci. Earlier workers attached some importance to the length of chains formed and differentiated a supposedly more virulent *Streptococcus longus* and a less virulent *Streptococcus brevis*. Other morphological and biochemical characteristics were thought to be associated. It has long been clear, however, that this distinction has little meaning in spite of the fact that freshly isolated streptococci from pathologic processes usually form chains of more than eight cells while those normally present in the mouth and throat usually develop only short chains. For instance, longer chains are generally formed during growth in liquid media; *Streptococcus lactis*, a common inhabitant of milk, forms very long chains; and short-chain streptococci are not infrequently isolated from pathologic conditions. The formation of chains of cells is in no sense absolute, however, and on microscopic examination of a smear of typical streptococci, single cells, pairs of cells, and occasional aggregates resembling staphylococci are found.

Streptococci are not motile under ordinary conditions of observation, but motile forms are described with some frequency, and the precise status of their motility is not clear. The majority of strains are encapsulated, and in some the capsular material consists of hyaluronic acid, the substrate of hyaluronidase or invasins (p. 212). So far as is known, these bacteria do not form spores, and the formation of pigment is relatively rare.

They stain readily with the usual bacterial stains. Almost all of the strains isolated from pathologic processes in man are gram-positive, but gram-negative streptococci are found with some frequency, more commonly in suppurative conditions in lower animals than in man.

Streptococcus colonies on agar media are usually quite small, translucent, convex, entire, and slightly granular, but colonial differences among variants are well known (*vide infra*). The growth on agar plates may be confluent if too heavily inoculated, but these bacteria have a tendency to remain in discrete colonies.

Physiology. As a group, the streptococci grow over a relatively wide temperature range, 10° to 42° C. Those of the pyogenic group, which is made up of human and animal parasites, have an optimum at 37° C. and are relatively restricted in range, those of the lactic group grow over 10° to 37° C., and the viridans group grows from 37° to 42° C. and includes one thermophilic species which grows at 50° C. The majority of streptococci are facultative anaerobes, but there are a few obligate anaerobic varieties.

The streptococci are among the more fastidious of bacteria with respect to nutritive requirements. They will usually not grow on meat extract media, and growth is ordinarily poor even on infusion media but may be somewhat improved by the inclusion of phosphate buffer (M/30) and a small amount, perhaps 0.1 per cent, of glucose. For the most part, however, infusion media enriched by the addition of 10 per cent defibrinated blood, ascitic fluid and similar substances are used and a medium such as blood agar is completely

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satisfactory for routine culture of the pathogenic forms. Many strains are hemolytic on blood agar, some showing the clear zones of β hemolysis (see Fig. 47), and others a zone of greenish discoloration or α hemolysis indistinguishable from that produced by the pneumococcus (Fig. 52). Growth also occurs in milk which is curdled by some species due to the fermentation of lactose. *Streptococcus* cultures may be preserved in serum broth or agar or infusion media gelatin slabs in the refrigerator.

The relatively complex nutritive requirements of these bacteria have been defined to a considerable degree. Most strains require glutamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid and biotin, together with thirteen or fourteen amino acids. According to Wilson¹ the streptococci of group A also require nucleic acid derivatives. Some strains have been cultivated in chemically defined media² and Bernheimer and Pappenheimer³ have developed a hydrolyzed casein medium supplemented with amino acids, salts, glucose and vitamins of the B group which supports good growth of hemolytic streptococci.

A wide variety of sugars is fermented and a number of polysaccharides are hydrolyzed. The chief fermentation product of glucose is lactic acid, and small amounts of formic and acetic acids and ethyl alcohol are formed.⁴ The hydrolysis of sodium hippurate and polymers such as inulin, starch and dextrin has some differential significance, together with the fermentation of lactose, sorbitol, glycerol, mannitol, maltose, sucrose and raffinose. Some strains liberate relatively large amounts of ammonia from peptone and this characteristic also has some differential value. With rare exceptions, inulin is not fermented nor are the streptococci dissolved in ox bile or a 10 per cent solution of bile salt; these characteristics have considerable practical importance in that they serve to differentiate the α hemolytic or green streptococci from the pneumococci.

The Formation of Toxic Substances. The streptococci form no endotoxin, i.e., the cell substance is only mildly toxic on parenteral inoculation, and no true exotoxin unless the scarlatinal toxin be included in the exotoxins. A number of toxic substances of agerisin-like character which contribute to the invasive qualities of the pathogenic streptococci are found in culture fluid. These include hemolysins, leucocidin, fibrinolysin, hyaluronidase and the erythrogenic or scarlatinal toxin, and there is, in addition, a substance lethal for mice whose relation to the other toxic substances is not clear.

Two kinds of filterable hemolysin produced by streptococci were described by Todd.⁵ They differ in that one, called *streptolysin S*, is sensitive to treatment with heat or acid, and the other which is designated *streptolysin O* is inactivated by oxygen, i.e., is inactive in the oxidized state, and the activity may be re-

¹ Wilson. *Proc. Soc. Exp. Biol. Med.*, 1945, 58:249.

² Hutchings and Wooley: *Science*, 1939, 90:41, Mellwain *et al*: *Biochem. Jour.*, 1939, 33:223, Hutner: *Jour. Bact.*, 1938, 35:429, King, Gary and Farrell: *Jour. Bact.*, 1938, 36:837, Subbarow and Rane: *Jour. Amer. Chem. Soc.*, 1939, 61:1616, Wooley: *Jour. Exp. Med.*, 1941, 73:487.

³ Bernheimer and Pappenheimer. *Jour. Bact.*, 1942, 43:481, 495.

⁴ Friedmann: *Jour. Bact.*, 1938, 35:527, *ibid.*, *Jour. Biol. Chem.*, 1939, 130:757, Smith and Sherman: *Jour. Bact.*, 1942, 43:725.

⁵ Todd: *Jour. Path. Bact.*, 1936, 47:423, Herbert and Todd: *Brit. Jour. Exp. Path.*, 1944, 25:242.

CHAPTER 60

LEPROSY, RAT LEPROSY, SARCOIDOSIS, AND JOHNE'S DISEASE

LEPROSY

History and Epidemiology.—Leprosy is a disease of great antiquity; when or where it originated is not known, but according to Rogers and Muir (1925) it is probable that the original focus was in the northern belt of Central Africa. The disease was described in Asia Minor about 345 B.C.; thence it spread gradually westwards, till in the Middle Ages practically every country in Europe was affected. During the fourteenth and fifteenth centuries leprosy in Europe declined rapidly; about the same time, however, it was carried to the Western Hemisphere. During the last 70 years the disease has appeared in Oceania, where it has spread widely; in Hawaii, for example, it was estimated in 1909 that 1 in every 40 natives was a leper (Brinckerhoff and Moore 1909).

At the present time the highest incidence of the disease is confined to tropical countries, particularly (1) Equatorial Africa; (2) the long belt stretching from Assam and Burma, through the Malay States and Indo-China to the islands of the Pacific; and (3) the West Indian Islands and the northern part of South America. Males appear to be more commonly affected than females. Infection seems to be most frequent in youth and early adult life; Rogers and Muir (1925) state that probably over 50 per cent. of patients are infected before the age of 20. The lower social classes are more frequently infected than the higher. The incubation period may be almost any time, from a few weeks to several years; the commonest period is 2 to 4 years, but it is said that it may extend even to 40 years. The disease is extremely chronic and may be of lifelong duration; after it has lasted for a variable length of time it may retrogress as the result of a naturally acquired immunity; on the other hand, after remaining chronic for years, it may take on an acute course. The mortality from the disease itself is comparatively low.

The way in which leprosy is conveyed is uncertain. Most workers are agreed that it is infectious, but the manner in which infection is spread is still largely a matter of conjecture. Considering the long incubation period of the disease, this is not surprising. The restriction of the lesions to the skin suggests that the bacilli gain entrance through cuts, abrasions, and fissures, but it is possible that the nasal mucosa may be primarily infected. Lepers suffering from the lepromatous form are very much more infective than those suffering from the anæsthetic form; this is because lepra bacilli are numerous in the nodules of the skin, but are uncommon in the anæsthetic patches. Blood-sucking flies and other insects have been suspected as carriers of infection, but no convincing evidence against them has been brought forward. On the whole the infectivity of the disease

stored by treatment with mild reducing agents such as sulfite. Both hemolysins are extremely labile at 37° C. and disappear rapidly after the first few hours of incubation. There is some evidence that the leucocidin of streptococci is identical with streptolysin O. The latter has been studied in some detail by Bernheimer,⁶ who has found that it has a cardiotoxic action on the isolated frog heart, and appears to be associated in some manner with the mouse lethal factor. The fibrinolysin (p. 211), acting as an activator of an inactive serum protease, is possibly associated with the invasive character of the pathogenic streptococci in facilitating the spread of infection through the barrier of fibrin clots.

The relation of hyaluronidase (p. 212) to the invasive properties of the streptococci is less clear than once thought. In general, as with many other pathogenic bacteria, the presence of capsules is associated with virulence and among the streptococci the capsular substance is frequently hyaluronic acid. The addition of hyaluronidase to suspensions of encapsulated streptococci thus denudes them of capsules and they are more readily phagocytosed and less virulent.⁷ This factor, then, assumes an anomalous position with regard to its contribution to the invasive properties of streptococci.

The erythrogenic toxin is a substance which gives rise to a marked local erythema upon intradermal inoculation in man, and in larger amounts produces a generalized erythematous rash. A skin reaction may be produced in the rabbit but in general laboratory animals are highly or completely resistant to it. The lethal dose for the rabbit is very large, 5 to 10 ml. of unconcentrated filtrate. This toxin is responsible for the rash of scarlet fever and is known as scarlatinal toxin, or Dick toxin after its discoverers. It differs from the classic exotoxins in that it is relatively heat-resistant and some toxicity still remains after boiling for thirty minutes (see also p. 375). It is antigenic and stimulates the production of specific antitoxin but not to the high titers readily obtained for diphtheria and tetanus antitoxin.

The formation of the foregoing substances by the streptococci is a characteristic of the group rather than of all strains of pathogenic streptococci. Thus all group A streptococci do not produce erythrogenic toxin, and those that do not are incapable of causing scarlet fever. In general they are associated with virulence in that avirulent varieties are frequently non-hemolytic, non-fibrinolytic and the like while those found in pathologic conditions and showing high virulence under experimental conditions are hemolytic, fibrinolytic, etc.

Another factor associated with the ability of streptococci to produce disease is the development of a hypersensitivity to the cell substance of these bacteria during and following infection. Subsequent infections, then, result in allergic phenomena which may be of very considerable importance in the disease produced. Thus, it seems probable that hypersensitivity plays a part in rheumatoid disease and arthritis (*vide infra*) of streptococcal etiology.

Variation. Alterations in the morphology of individual streptococci are frequently observed in old cultures, with cells swollen to several times normal size. These and other changes in aging cultures have been interpreted by some workers as indicative of a complex life cycle, but it is more likely that

⁶ Bernheimer and Cantoni. Jour. Exp. Med., 1945, 81: 295, 307. *ibid.*, 1947, 86: 193.

⁷ See Kass and Seastone. Jour. Exp. Med., 1944, 79: 319.

natural infection of the Syrian hamster with the rat leprosy bacillus has been ascertained, it would be rash to do more than suggest that this organism may be responsible for occasional cases in man.

Diagnosis.—In the lepromatous (tubercular) form, a piece of skin over one of the nodules should be examined microscopically for leprosy bacilli (Fig. 282). With a sharp pair of scissors curved on the flat, a piece of skin 3 mm. long and 2 mm. deep is clipped off the surface. The corium is pressed downwards on to a clean new slide, and a film preparation made, and stained with Ziehl-Neelsen (Rogers and Muir 1925); or the skin may be fixed, embedded in paraffin, and sections cut in the usual way. If acid-fast bacilli cannot be found in the nodules, it is advisable to examine the nasal mucosa. The most satisfactory method is to scrape the mucosa gently with a blunt scalpel, and make ordinary film preparations. The bacilli can frequently be demonstrated in this situation in the lepromatous form, and may be found in some cases of the maculo-anæsthetic form of the disease. Sometimes they are present in the nasal mucosa before skin nodules have developed; thus they are said to be demonstrable occasionally in apparently healthy contacts. In early cases of generalized leprosy, the bacilli can often be demonstrated in clippings from the lobule of the ear. If there is any doubt as to whether the organisms found in the nose or skin are true leprosy bacilli, it is advisable to inject a suspension of the ground-up material into guinea-pigs; if they are leprosy bacilli, there will be no result; if on the other hand they are tubercle bacilli, the animals will develop the usual form of experimental tuberculosis.

When the lungs are affected, the sputum may contain lepra bacilli, which are most easily distinguished from tubercle bacilli by animal inoculation.

The complement-fixation test has been employed for the diagnosis of leprosy, but it does not appear to be of much value in practice, since a positive reaction may be obtained with antigens made from tubercle bacilli (Row 1925-26).

Lepers frequently give a positive Wassermann reaction; doubtless this is often due to the co-existence of syphilis, but there appears to be little doubt that the Wassermann reaction may be positive in leprosy in the complete absence of syphilitic infection. In countries in which the positive Wassermann reaction rate varies from 7 to 20 per cent amongst the general population, many observers have found that the rate amongst lepers varies from 15 to 95 per cent. (Rogers and Muir 1925). The great variation in the percentage of positive reactions amongst the leprosy population seems to depend largely on the type of antigen employed. Thus Hasseltine (1924) examined the serum of 236 lepers in Hawaii. Using an acetone-insoluble antigen he obtained 17.8 per cent. of positive results; with an antigen consisting of an alcoholic extract of beef heart to which 0.4 per cent. of cholesterol had been added, he obtained 50 per cent. of positives; and with Kolmer's (1922) new antigen he obtained 21.6 per cent. of positives. The greatest number of positive reactions occurred in lepers with the nodular type of



FIG. 282.

Leprosy bacilli in the tissues. The bacilli are seen in dense clumps, mostly intracellular ($\times 1000$).

such aberrant morphology is that of involution forms, *i.e.*, is degenerative in nature.

Dissociative changes in colonial morphology are well known and have been described by Todd* and by Dawson, Hobby and Olmstead.⁴ It was shown by the former that a form designated as matt is the virulent form and distinct from the usual smooth and rough colony types. The latter workers described a mucoid colony type which was still different. There are, then, four recognized colonial types: smooth, rough, mucoid and matt. The conversion from mucoid or matt to rough or smooth corresponds to the usual S→R dissociative change. The type-specific M protein is present in all but the rough form. Other immunological variation, not correlated with colonial form, may occur also, for there is some evidence that the agglutinative types are sometimes unstable, and that on occasion the group-specific polysaccharide may be lost.

Variation in hemolysis is commonly reported in which β hemolytic strains give rise to non hemolytic or α hemolytic variants. The alteration in hemolysis is to some degree an environmental effect in that anhemolytic variants may be hemolytic under anaerobic conditions, suggesting an inactivation of oxygen-labile hemolysin rather than failure to produce it; similarly α hemolytic variants may be made β hemolytic by including catalase in the medium or omitting reducing sugar since the latter appears to inhibit hemolysin production by some strains of streptococci. Streptolysin O is generally regarded as responsible for blood plate hemolysis, but anhemolytic variants of β hemolytic strains have been observed which continue to form streptolysin O in liquid culture.

Streptococci, like other bacteria, may become resistant to the action of chemotherapeutic drugs in the laboratory. Drug fast strains may also be found in naturally occurring infections but whether these arise by a process of selection or by *in vivo* adaptation in the presence of drugs during therapy is not clear (p. 178). Whatever the mechanism, the widespread, and often indiscriminate, use of such drugs through the general availability of sulfathiazole tablets, penicillin tablets and the like, has been regarded by many as undesirable because of this possible consequence. The development of just such a situation occurred during World War II through the use of sulfadiazine as a prophylactic for the control of streptococcus infection in naval training camps.¹⁰ The sequence of events was as follows. Since preliminary studies indicated that streptococcal infection among trainees could be controlled by prophylactic sulfadiazine, all personnel at one training center were placed on such a prophylactic regime on March 1, 1944. Following its institution streptococcal infection increased but this was associated with a country wide increase and tests made at the time indicated that no sulfa fast streptococci were present at the center. At that time *Str. pyogenes* Type 19 was responsible for about 28 per cent of the infections. The high incidence of infection continued and by May Type 19 was responsible for 95 per cent of cases of streptococcal disease. At that time it was found that a high percentage of strains of Types 19 and 17 isolated were sulfa fast, and Type 3 had also become drug fast. These drug fast strains were

* Todd, *Brit. Jour. Exp. Path.*, 1925, 9:91, *Jour. Exp. Med.*, 1932, 55:267.

⁴ Dawson, Hobby and Olmstead, *Jour. Inf. Dis.*, 1939, 62:139 also Morton and Seem, *Jour. Bact.*, 1944, 47:123.

¹⁰ *Jour. Amer. Med. Assn.*, 1945, 129:921, *Damrosch, et al.*, 1946, 130:124.

with tuberculosis protects against leprosy, or in fact that there is any appreciable degree of cross-immunity between the two diseases. It will be surprising therefore if vaccination with B.C.G. has any effect on the incidence of leprosy (see Lowe and McNulty 1953).

Though chaulmoogra oil was employed for many years in treatment, it has now been displaced by the sulphones. Very good results were reported by Lowe (1950) from the oral administration of diamino-diphenyl sulphone. Of 50 patients treated for more than six months, 36 showed clinical improvement, 31 bacteriological improvement, and 3 became bacteriologically negative. In the treatment of rat leprosy Cruickshank (1951) found that isoniazid prolonged the life of the animals but did not prevent ultimate death.

RAT LEPROSY

Rat leprosy is a disease that was first described by Stefansky (1903) in 1901 at Odessa. Rats were being slaughtered in large numbers, consequent on the outbreak of human plague, and rat leprosy was found in 4-5 per cent. of them. The incidence reported by other observers varies considerably.

In the United States McCoy (1913) found it in 186 out of about 200,000 rats caught in San Francisco, Cal., an incidence of 0.093 per cent.; and Wherry (1908) found it in 20 out of 9,631 rats caught in Oakland, Cal., an incidence of 0.21 per cent. In Japan, Ota and Asami (1932) found it in 0.7 per cent. of *Rattus norvegicus* and in 0.1 per cent. of *Rattus rattus*; and Yamamoto, Sato and Sato (1936) found it in 1.24 per cent. of 2,573 rats caught in Tokyo. According to Marchoux (1933), about 5 per cent. of the sewer rats in Paris harbour rat leprosy bacilli in the lymph nodes, but in only about 0.6 per cent. of the rats are generalized lesions present. In Batavia 9.3 per cent. of about 10,000 rats were said by Lampe and de Moor (1936) to have lesions of the lymph nodes, but not more than 0.23 per cent. of the animals had manifest skin leprosy.

The disease exists in two forms—the glandular type and the musculo-cutaneous type—but there appears to be no sharp division between them. In the glandular type one or more of the groups of subcutaneous glands—inguinal, axillary, or cervical—is enlarged, hard, and of whitish colour. On section the glands are uniform and hard; there are no nodules or necrotic areas visible macroscopically. Microscopically, the capsule and trabeculae are thickened; the sinuses are filled with dense aggregations of irregularly polygonal cells, which have a large nucleus and much cytoplasm—probably macrophages; the cytoplasm is packed with acid-fast bacilli, so that the contours of the cell-body are often invisible. A few giant cells, with several peripheral nuclei, containing numerous bacilli in their cytoplasm are also visible. Some organisms may be found free.

In the musculo-cutaneous type the rat is emaciated; the skin presents one or more irregularly round or oval areas of alopecia, commonest on the head; occasionally ulcers are seen, about 0.5 cm. in diameter, covered with a mealy-looking discharge containing acid-fast bacilli. At the site of these areas of alopecia the skin is atrophic. The subcutaneous tissues are devoid of fat, and show the presence of a greyish-white, granular material, which Stefansky regarded as altered muscle tissue; it contains numerous acid-fast bacilli. Sometimes nodular masses are found in the muscles, covered with particles of stretched atrophic skin. Stefansky believed that the primary lesion begins in the subcutaneous muscle fibres, and sometimes spreads to the skeletal muscles. Histologically, numerous acid-fast

highly communicable and formed scarlatinal toxin. The drug-fast Type 19 appeared at other training centers coincident with the transfer of personnel. By July prophylactic sulfadiazine had been discontinued in all but two of five primary training centers where it was retained for half the personnel for experimental purposes. Subsequent analysis showed that these two centers had the highest admission rates for patients with throat cultures positive for hemolytic streptococci, and the incidence of scarlet fever was much higher in the treated groups. Under the circumstances prophylactic sulfadiazine apparently increased the incidence of streptococcal infection, and it was suggested that the drug-fast strains were actually stimulated by small amounts of the drug or that the drug inhibited selectively normal flora of the throat and nose which ordinarily had some antibiotic effect on streptococci. Not only did the drug-fast strains spread through personnel from one locality to another,

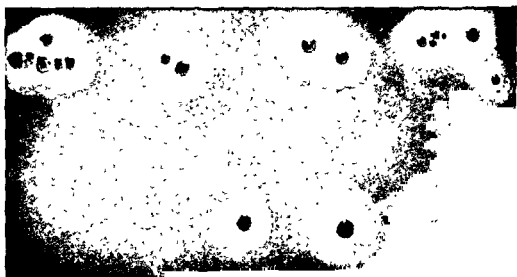


Fig. 47. *Streptococcus pyogenes*. Pure culture on blood agar showing β hemolysis. $\times 5$.

but drug-fast Type 19 *Str. pyogenes* appeared in the civilian population coincident with the return of large numbers of armed service personnel to civilian life.¹¹ It will be clear that the development of resistance to chemotherapeutic drugs can be a matter of very considerable practical importance, especially with virulent pathogenic bacteria as ubiquitous as the streptococci.

Classification.¹² The differentiation and identification of the streptococci is a matter of very considerable practical importance because of their etiological relation to a number of widespread diseases of man and domestic animals, and of equal importance from the theoretical point of view. It has been and continues to be a particularly difficult matter since neither the physiological nor immunological methods have been satisfactory. As a consequence there is basic disagreement among workers in this field as to what constitutes a species or variety and on what basis or bases differentiation should be made. Three general criteria have been used, viz., hemolysis on blood agar plate culture, biochemical properties and immunological character as indicated by precipitin and agglutination reactions. Those concerned with the pathogenic streptococci

¹¹ Johnson and Hartman. *Jour. Clin. Invest.*, 1947, 26:325.

¹² See Sherman. *Bact. Rev.*, 1937, 1:1, *Jour. Bact.*, 1938, 35:81.

Sellards and Pinkerton (1938) showed that progressive lesions could be readily reproduced by the intracerebral inoculation with rat leprosy bacilli of rats and mice, less readily of monkeys (*M. rhesus*) and rabbits, but not of guinea-pigs. In mice the infection can be reproduced serially by simple intraperitoneal inoculation.

Balfour-Jones (1937) found that the disease could be transmitted to the Syrian hamster (*Cricetus auratus*). Intraperitoneal inoculation with 0.25 ml. of a suspension of infected material from a rat caused death in about 6 months. The picture at post-mortem was similar to that in rats, except that the omentum was less thickened. Lesions were commonly present in the liver and spleen, less often in the kidneys and lungs. When interstitial nephritis was observed, clumps of acid-fast bacilli were found in the interstitial tissue. In rats, it may be noted, acid-fast bacilli are never found in the kidney. Subcutaneous inoculation also produced lesions in the hamster.

SARCOIDOSIS

This disease is mentioned only because of its possible relationship to tuberculosis and leprosy. Nothing certain is known about its ætiology, and there is still doubt as to whether it is of infectious origin. Though the first recognizable manifestations are usually in the skin—cutaneous sarcoids—other organs are often affected, such as the lymph nodes, lungs, liver, bones, and eyes. Pathologically, the reticulo-endothelial system is affected; the lesions are characterized by aggregations of epithelioid cells, with giant cells but little or no necrosis. The course of the disease is chronic and local healing may occur. Acid-fast bacilli cannot be found in the lesions, nor has any organism of possible pathogenic significance been isolated from them. Of special interest is the fact that a higher proportion of patients than of normal persons react negatively to the tuberculin test. According to Wells and Wylie (1949), this is because, in certain stages of the disease, a tuberculin-neutralizing factor is present in the blood. (For review of the disease, see Michelson 1948.)

Lofgren and Lundback (1950) reported the isolation of a filtrable virus from six cases of the disease. Fluid from gastric lavage was inoculated into the allantoic and amniotic cavity of the fertile egg. After 3 or 4 "blind" passages it was found that the allantoic fluid agglutinated the red blood corpuscles of fowls, sheep, guinea-pigs, and men, and gave a positive complement-fixation reaction with serum from the patients. The agent passed through a gradocol membrane of 250 $m\mu$ but not of 160 $m\mu$ A.P.D. Injected intranasally and intracerebrally into cynomolgus monkeys, it gave rise to fever in 4-7 days, and blood taken during the febrile stage and passed twice through eggs gave rise to hæmagglutinins in the allantoic fluid. The authors regard the agent as belonging to the influenza-Newcastle group of viruses, resembling the mumps virus most closely. These findings await confirmation.

JOHNE'S DISEASE

SYNONYMS.—*Enteritis chronica pseudotuberculosis bovis*; *Entérite spécifique chronique des bœufs*; *Chronischer infektiöser Darmkatarrh des Rindes*; chronic bovine pseudo-tuberculous enteritis.

John's disease is a specific enteritis affecting cattle, and less frequently sheep and deer, caused by an acid-fast organism known as John's bacillus. It was first described by John and Frothingham in 1895 at Dresden. They regarded it as a peculiar form of tuberculosis, possibly due to the avian type of bacillus.

The disease is fairly common in Northern Europe, and has been observed in

make a tentative preliminary separation on the basis of hemolysis, and define species and types on an immunological basis. Workers with more general interests tend to rely primarily on physiological characters, and this is the basis of the Bergey (1948) classification.

Hemolysis. The use of blood plate hemolysis was introduced by Schottmuller in 1903 and is especially convenient since blood agar is the medium of choice in primary isolation. On this basis three types of streptococci may be distinguished:

- (1) The β hemolytic streptococci which produce a clear zone of hemolysis in the red, opaque medium immediately surrounding the colony.
- (2) The α hemolytic or green streptococci, which produce a zone of greenish discoloration in the medium about the colony which is considerably smaller than the clear zone of β hemolysis.
- (3) The anhemolytic, indifferent, or γ streptococci which produce no change in the medium.

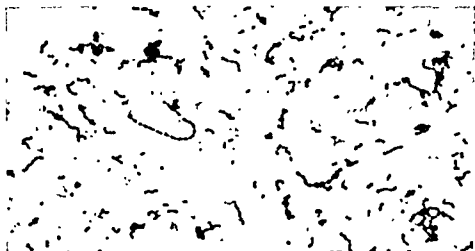


Fig. 48. *Streptococcus fecalis*. P strain isolated from focal infection. Smear from pure culture Fuchsin, $\times 1050$.

These distinctions have some validity in that the highly virulent streptococci isolated from pathologic conditions are almost invariably the β hemolytic variety. In the older literature these are grouped as a single species with the name *Streptococcus hemolyticus*, and some workers further differentiated on the basis of the disease with which the strain was associated, viz., *Streptococcus scarlatinae* (scarlet fever), *Streptococcus epidemicus* (epidemic septic sore throat), *Streptococcus erysipellatis* (erysipelas), etc. It is now quite clear that these distinctions are invalid in that identical streptococci may cause more than one clinical disease, and that the same disease may be caused by immunologically distinct streptococci; that is to say, there appears to be no basis for the concept of disease-specific types of streptococci, so far as diseases of man are concerned. The β hemolytic streptococci associated with diseases of lower animals do, however, show a high degree of specificity in the case of *Streptococcus equi*, causing strangles in horses, and *Streptococcus agalactiae*, causing mastitis in cattle, but not in that of the organism now known as *Streptococcus*

sub-mucosa, and very occasionally the underlying muscle layer. The bacilli are arranged in dense clumps, and may be intra- or extracellular. Johne and Frothingham were of the opinion that they were primarily intracellular in position, but that owing to the subsequent disintegration of the cell, they were set free in the tissue. The cellular reaction around the bacilli is diffuse, not localized as in tuberculosis. Bacilli can generally be found microscopically in the mesenteric glands. By cultural methods they can be shown to be widespread throughout the body, being distributed in the liver, spleen, lung, and many of the lymph nodes in the cervical and abdominal regions (Taylor 1951). In sheep there may be very much less thickening and corrugation of the intestinal mucosa than in cattle. The disease can be reproduced in cattle and sheep by feeding or parenteral inoculation with pure cultures of Johne's bacillus (see Chapter 16).

The bacteriology of the disease is described in Chapter 16.

Diagnosis and Prevention.

losis, c
found in very small numbers in the faeces (McFadyean *et al.* 1912), but they cannot be distinguished microscopically from saprophytic acid-fast bacilli. For diagnostic purposes it is better to look for them in a piece of rectal mucosa removed with forceps or a Volkmann's spoon. If they are found, the infected tissue should be injected into a guinea-pig to exclude tuberculosis. The organisms may be cultivated from the faeces by the method described on p. 529, but the method is too cumbersome for routine use. For culture from the tissues the oxalic acid method of treatment followed by inoculation on to 4 per cent. glycerol egg yolk medium containing 1 per cent. of heat-killed *Mycophleia* is recommended by Taylor (1933). Sigurdsson (1945, 1946, 1947) reported favourably on a complement-fixation test in sheep; though useless for latent infections, it appears to be of value for confirmatory purposes. The tuberculin test with bovine tuberculin is negative, but a reaction often follows the injection of avian tuberculin (see Bang 1909, 1914). A substance called *johnin*, resembling old tuberculin, and prepared from glycerine broth cultures of Johne's bacillus, was introduced by McFadyean, Sheather and Edwards (1912) and Twort and Ingram (1913). (See also Dunkin 1933.) The intradermal injection of this substance often gives rise to a reaction in infected animals which, in the absence of a reaction to tuberculin, is very suggestive; but the test is not strictly specific. Thus, naked-eye evidence of disease may be absent at the post-mortem examination of animals reacting to *johnin*. This is not surprising in view of the frequent occurrence of latent infection revealed only by cultural methods. What is more convincing is that gross lesions may be found in negative reactors (see McEwen 1939, Report 1941). In doubtful cases of infection at post-mortem, the best method is to culture the ileo-caecal glands (Taylor 1951).

The disease is usually fatal, but numerous instances of apparent recovery or of long intermissions in its course are known. In some of these animals post-mortem examination has revealed the presence of intense cellular reactions in the mesenteric glands, which had apparently resulted in the disappearance of the bacilli. It seems probable that in these animals infection had remained latent and never given rise to clinical symptoms.

Since the disease appears to be spread mainly by the contamination of water, foodstuffs and pasture land with the faeces of infected animals, preventive measures will include the destruction of diseased animals, suitable disposal of their excreta, disinfection of byres, the ploughing-up of infected pasture, and the segregation

zooepidemicus which causes a wide variety of suppurative diseases in animals, including mastitis in cattle. The β hemolytic streptococci of human disease are usually not found in lower animals, but may occasionally infect the udder of the cow, giving rise to milk-borne septic sore throat. Conversely, those of animal origin do not ordinarily infect man, though human infections with *Str. zooepidemicus*, while not common, are somewhat more so than is generally believed. An even greater heterogeneity in the group is indicated by the fact that some non-pathogenic forms are also β hemolytic. It will be quite clear, therefore, that the inclusion of all these forms under the single species *Str. hemolyticus* is hardly justified.

A somewhat similar situation holds true with the α hemolytic or green streptococci which have been grouped as a single species, *Streptococcus viridans*. The green-producing group embraces such forms as the fecal streptococci or enterococcus group, those which normally inhabit the mouth and throat, and non-pathogenic forms. Some of the α hemolytic forms, especially those found in the throat and intestine, are able to set up disease processes if normal resistance is reduced, with the production of localized infections at the roots of teeth, in the heart valves in bacterial endocarditis, etc. These pathogenic forms differ rather sharply from the highly virulent β hemolytic streptococci, but again the entire group is too heterogeneous to justify inclusion in a single species.

The anhemolytic or indifferent streptococci are almost all saprophytic forms found in milk and various dairy products. The only pathologic condition with which they have been unequivocally associated is subacute bacterial endocarditis,¹³ in which they have been found in a small minority of cases. Here a variety of physiologically different types are included in a single group, and, as in the case of the other groups separated on the basis of hemolysis, it is too heterogeneous to allow lumping into the single species, *Streptococcus anhemolyticus*.

Immunological Differentiation. Largely through the work of Lancefield and her co-workers the pattern of antigenic structure of the β hemolytic streptococci has been defined, but the viridans and anhemolytic forms are serologically diverse so that this approach has not been useful. By the extraction of soluble antigens and application of the precipitin test, Lancefield¹⁴ has demonstrated the presence of both group-specific and type-specific antigens in the hemolytic streptococci. The group-specific antigen, or "C" substance, is polysaccharide in nature and an integral part of the bacterial cell rather than a capsular material. On the basis of the specificity of this antigen five groups were originally described and designated Group A, Group B, and so on, additional groups have since been found up to and including Group N. The biological significance of these groups is indicated by the origin of the strains making them up, viz.:

- Group A—Primarily pathogens of man
- Group B—Found almost entirely in mastitis of cattle
- Group C—Primarily pathogens of lower animals
- Group D—Found in cheese
- Group E—Found in milk

¹³ See the discussion of this group by Rosebury *Medicine*, 1944, 23:249.

¹⁴ *Jour Exp Med.*, 1925, 42:377, 397, *ibid.*, 1928, 47:91, 469, 481, 483, 857, *ibid.*, 1933, 57:571.

The association of origin and immunologic group is, however, not absolute. Streptococci of Group A are occasionally found in lower animals, producing mastitis in cattle for example, while those of Group C are found in man with some frequency as indicated in the accompanying table.

INCIDENCE OF STREPTOCOCCI OF GROUPS A AND C IN HUMAN INFECTIONS*

Type of Infection	Number of Strains	Group A		Group C	
		Number	Per cent	Number	Per cent
Scarlet fever	232	229	98.7	3	1.3
Tonsillitis and septic sore throat	52	52	100.0	0	0
Rheumatic fever and rheumatic arthritis	19	19	100.0	0	0
Puerperal sepsis	55	51	92.7	4	7.3
Erysipelas	51	48	94.1	3	5.9
Miscellaneous	143	136	95.1	7	4.9
Total	552	535	96.9	17	3.1

* From Evans Jour. Bact. 1944, 48:267

Type specificity among the streptococci was indicated by the work of Griffith¹⁵ who separated β hemolytic streptococci isolated from human disease into 27 types by slide agglutination, and later added three more types. These were given arbitrary arabic numbers, viz., Type 1, Type 2, etc. Following definition of the serological groups of Lancefield, it was found that these types are distributed over the groups, Group A included the majority, 23 in all, and Types 7, 20 and 21 fell into Group C, and Type 16 into Group D.

Lancefield has shown that the types within Group A are determined by two type-specific antigens. One of these, the "M" antigen, is a nucleoprotein which is destroyed by proteolytic enzymes, and may in fact be digested by a soluble protease produced by streptococci,¹⁶ resulting in strains which cannot be typed by "M" antisera. The other type-specific antigen, designated the "T" antigen, is not as well known biochemically, it is resistant to proteolytic enzymes, but more recent work¹⁷ suggests that it is also protein in nature. The "M" and "T" antigens are independent and may occur in various combinations. Thus, Types 10 and 12 contain the same "M" antigen but different "T" antigens, while Types 15, 17, 19, 23 and 30 contain very closely related "T" antigens but distinct "M" antigens. In addition, strains are found which lack

¹⁵ See Griffith Jour. Hyg., 1935, 35:23

¹⁶ Elliott Jour. Exp. Med., 1945, 81:573

¹⁷ Lancefield and Dole Jour. Exp. Med., 1946, 84:449.

"T" antigen, and still others lack, or apparently lack, "M" antigen. While, then, the separation of the streptococci of Group A is clearly a complex matter, streptococcus typing has been rather generally applied, in part because it is of importance in the interrelationships, possibly phylogenetic, of the streptococci, and in part because it is of great value in epidemiological studies. Many workers feel that slide agglutination is not completely satisfactory and favor the precipitin reaction¹⁸, a method of carrying out the precipitin test in capillary tubes which is reliable and conserves serum has been developed. A detailed discussion of typing methods is given by Stewart, Lancefield, Wilson and Swift.¹⁹

In general these types are regarded as quite stable, but Evans²⁰ is of the opinion that they are not completely so and has reported that shifts in type occur on storage of cultures and as a result of animal passage. In this connection, Wilson²¹ has found that a strain of Type 27 lost its group specificity, the "C" substance, during mouse passage, though type-specific precipitinogens were retained.

STREPTOCOCCAL ANTIGENS*

Group Specificity		Type Specificity		
Group	Antigen	Number of Types	Antigens	
			Name	Nature
A	"C" substance (polysaccharide)	More than 30	"M" "T"	Nucleoprotein Probably protein
B	"C" substance (polysaccharide)	4 main types plus subtypes	"S"	Polysaccharide
C	"C" substance (polysaccharide)	Possibly 13	No symbol	Protein
D et seq	"C" substances (polysaccharides)	Several	"S"	Polysaccharides

* Modified from Lancefield Harvey Lectures, 1940-41, Ser 36, pp 251-290

Streptococci of the mastitis streptococci, four main types and a number of subtypes have been differentiated. In this group there is but a single type-specific antigen, and it is polysaccharide in nature. Group C contains, in addition to the three Griffith types noted above, ten additional types, five in strains of human origin and

¹⁸ See Hilles and Hamburger: Jour. Inf. Dis., 1944, 75:265

¹⁹ Stewart, Lancefield, Wilson and Swift Jour. Exp. Med., 1944, 79:99.

²⁰ Evans: Jour. Inf. Dis., 1946, 78:204.

²¹ Wilson Jour. Exp. Med., 1945, 81:593.

the bacillus which he had isolated would produce lesions closely resembling those of human diphtheria. This demonstration was the logical extension of the results recorded by Trendelenburg in 1869 and by Oertel in 1871, both of whom had succeeded in producing false membranes in rabbits and pigeons by inoculating them with material from human lesions. Loeffler had further shown that the subcutaneous or intramuscular inoculation of cultures of the bacillus into susceptible animals was followed by death associated with lesions which, though bearing little superficial resemblance to human diphtheria, were in themselves highly characteristic; that these lesions were not the direct result of tissue invasion by the bacilli; and that certain animal species were highly resistant to infection by any route.

The next important step in our knowledge of diphtheritic infection was the discovery by Roux and Yersin in 1888 that sterile filtrates from cultures of the diphtheria bacillus would kill guinea-pigs with lesions identical with those which follow the injection of the living organism. This demonstration of the presence of a potent extracellular toxin afforded an explanation of Loeffler's observation that death was not associated with any spread of bacteria beyond the local lesion. In rabbits inoculated with such filtered cultures Roux and Yersin noted the occurrence of late paralysis.

The lesions produced in experimental animals have been described on pp. 544-6. It will be recalled that diphtheritic toxæmia in the guinea-pig is associated with fatty degeneration of the heart muscle and diaphragm and with congestion and hæmorrhage in the adrenal glands. In man there is reason to believe that the direct action of the toxin on the heart muscle is one of the chief causes of death (Bolton 1905, Dudgeon 1906, Jaffé 1920, Andrewes *et al.* 1923); adrenal changes are much less evident, though histological examination may reveal the presence of congestion with small extravasations of blood and degenerative changes in the cortical and medullary cells (Andrewes *et al.* 1923).

We have already referred to Loeffler's observation that the diphtheria bacillus can be recovered from the local experimental lesion, but not usually from the internal organs. It has generally been held that very little invasion of the body tissues occurs apart from the site of the local lesion. The organism has rarely been isolated from the blood stream during life; only 3 positive results appear in the records of 313 cases of diphtheria studied by Leede, Roedelius and Reiche in Hamburg (see Andrewes *et al.* 1923). According to Wildfuhr (1949), however, this is because cultures have been made too late. Wildfuhr himself, who took blood from 202 contacts of diphtheria patients, isolated virulent diphtheria bacilli from 31 of them up to three days before the appearance of the local membrane; in 3 of them bacilli were present in the blood at the time of appearance of the membrane and in only 1 of them after it had appeared. The patients were suffering from constitutional symptoms—fever, malaise, headache and nausea—and in most of them a slight swelling and redness of the tonsils could be detected. At autopsy, individual observers claim to have demonstrated the organism in various internal organs, but the only viscus from which it can commonly be cultivated is the lung—and this organ is more likely to be infected by direct inhalation than from the blood stream (see Report 1923).

The evidence, as a whole, clearly suggests that natural diphtheria in man, like experimental diphtheria in the guinea-pig, rabbit, or pigeon, is essentially a toxæmia in which individual resistance or susceptibility to infection, and recovery or death, are largely determined by the presence or rapid production of antitoxin. Tissue

While the antigenic structure just discussed is largely that of the β hemolytic streptococci, β hemolysis is not invariably associated with the immunological character of these groups, and it has been found that certain of the enterococci, for example, contain these antigens. By and large, however, this antigenic structure does not extend far beyond the β hemolytic group and, as indicated above, immunological methods have not been useful in other than this group.

Physiological characteristics have been used exclusively in the Bergey (1948) classification of the streptococci and the species differentiated on that basis are indicated in the following abridged key.

A Phyogenic group

a Lactose fermented

- b Lactose fermentation variable

2. Sodium hippurate hydrolyzed Lancaster Group B

1. Lactose fermented

- ²² The biochemistry of the streptococcal immunol., 1943, 47 513, and by Lancefield 979.

Reviewed by E. J. ...
1943, 78-465

cent., *intermedius* infections 4.27 per cent., *mitis* infections 20.11 per cent. Later information is afforded by Cooper and his colleagues (1936) and by W. T. Russell (1943), both of whom analyse the distribution of the types in several different centres in England and Wales, and by McLeod (1943), who includes data from many different countries. In general, the predominant strain is far more often of the *gravis* or *intermedius* than of the *mitis* type, suggesting that *mitis* diphtheria is usually endemic, whereas *gravis*, and to a less extent *intermedius*, diphtheria tend to be epidemic. Changes in distribution of the different types may occur from year to year (see Robinson and Marshall 1934, Wright 1941b, Report 1943). For example, in Manchester the *gravis* infections rose from 23.8 per cent. in 1934 to 50.9 per cent. in 1935; in Liverpool they rose from 34.2 per cent. in 1937 to 69.6 per cent. in 1940. It may be noted that in the United States some workers have failed to type their strains satisfactorily; the reasons for this cannot be discussed here (see Perry *et al.* 1936, Frobisher 1938, 1940, 1942, Seligmann 1941).

There is further evidence to show that the *gravis* and, to a less extent, the *intermedius* types are able to spread more readily than the *mitis* type in naturally immune or artificially immunized populations. What the factors are that determine the greater virulence of the *gravis* and *intermedius* types are still problematical. Early observations failed to reveal any differences in the virulence of the three different types for guinea-pigs, in the amount of toxin produced, or in the neutralizability of the toxins by antitoxin. Later observations, however, have tended to reveal small but possibly significant differences between them.

Povitsky, Eisner and Jackson (1933), for example, found the minimal lethal dose of *gravis* strains for the guinea-pig to be smaller than that of *mitis* strains; they also found that *gravis* strains were more effective in controlling mice than *gravis* infections. Murray and antitoxin against types. Gundel and König (1938) observed that, for protecting animals by active immunization against *gravis* strains, a qualitatively better antigen was required than against *mitis* strains. Gundel and Erzin (1936) noticed differences in guinea-pigs infected with standard doses of the three types; those infected with *gravis* strains died soonest, and those infected with *gravis* or *intermedius* strains yielded positive cultures from the internal organs more frequently than those infected with *mitis* strains. Again, Clauberg (1939), after examining a large number of toxins grown under standard conditions, reached the conclusion that *gravis* toxins were more potent in producing a skin reaction than *mitis* toxins and that their neutralization required more antitoxin. Finally, Orskov, Andersen, and Poulsen (1944) found that, when the bacilli were injected subcutaneously in a dose of 10 million into 150-gram guinea-pigs, the *gravis* strains proved fatal more rapidly than the *intermedius* or *mitis*, and at the site of injection the organisms were more abundant, there was less phagocytosis (see also Orr-Ewing 1946), and the edema fluid contained much more toxin.

Some observers have noted a relation between the iron content of synthetic media and the potency of the toxin produced (Pappenheimer and Johnson 1936, Happold 1940); and Mueller (1941) puts forward the interesting suggestion that in the human body, where iron is abundant, *gravis* strains may be the most potent toxin producers. Zinnemann (1943), who grew strains of different types in a broth medium having an iron concentration similar to that in the human tissues, obtained some evidence to support this suggestion. His further observations, carried out on the guinea-pig's skin, failed to reveal any difference in the nature of the toxins formed by the different types and led him to conclude that the greater severity of *gravis* and *intermedius* infections is due to the greater amount of toxin produced by these types in the body (Zinnemann 1946).

- Streptococcus salivarius**
- Streptococcus mitis*
- 11. Starch is hydrolyzed, bile tolerant
 - Streptococcus bovis*
- b. Grows at 50° C.
 - Streptococcus thermophilus*
- 2. Lactose not fermented
 - Streptococcus equinus*
- C. Lactic group
 - 1. Maltose +, dextrin +, ammonia from peptone
 - Streptococcus lactis*
 - 2. Maltose —, dextrin — (usually), no ammonia from peptone
 - Streptococcus cremoris*
- D Enterococcus group Lancefield Group D
 - 1. Not β hemolytic
 - a. Does not hydrolyze gelatin
 - Streptococcus fecalis*
 - b. Gelatin hydrolyzed
 - Streptococcus liquefaciens*
 - 2. β hemolytic
 - a. Mannitol +, sorbitol +
 - Streptococcus zymogenes*
 - b. Mannitol —, sorbitol —
 - Streptococcus durans*
- II Microaerophilic or obligate anaerobes
 - Streptococcus anaerobius*
 - Streptococcus foetidus*
 - Streptococcus putridus*
 - Streptococcus lanceolatus*
 - Streptococcus micros*
 - Streptococcus parvulus*
 - Streptococcus intermedius*
 - Streptococcus evolans*

Most of these species are generally accepted and it will be clear from the foregoing discussion that all members of Group A are known as *Str. pyogenes*, the immunological varieties within the group being types of *Str. pyogenes*. Two species are included in Group C. The name *Str. zooepidemicus* is a new one introduced in the Bergey (1948) classification, it does not replace another name but rather gives species status to the animal pathogens of this group formerly casually known as "animal pyogenes" which rarely if ever occur in man. The other species, *Str. equisimilis*, includes those streptococci formerly known as "human C" and which are uncommon in lower animals. Of the β hemolytic streptococci found in man, 95 per cent or more are Group A and therefore *Str. pyogenes*, and the remainder, Group C, are *Str. equisimilis*, differentiation being made by the precipitin test, using group-specific antisera. Of the green streptococci found in man, the most common are *Str. fecalis* of the enterococcus group and *Str. mutis* and *Str. salivarius* of the viridans group. So far as the etiology of streptococcal infections in man is concerned this differentiation, and that of *Str. fecalis* and *Str. liquefaciens*, is unimportant; in fact it is open to serious question as to whether the last should be regarded as other than a variant of *Str. fecalis*. The identification of streptococci which do not fall into

* May be confused with the pneumococcus because it ferments inulin, but, is distinguished by its lack of bile solubility.

It may be noted that the constant 0.0218 in the formula quoted above indicates that about 5 per cent. of those carrying on any one day have ceased to carry on the day following. In order to determine whether this rate of clearing held true in general, or applied only to this particular sample, figures collected from various published reports were analysed in the same way. It was found that the general form of the curve was very similar in each case, i.e. the logarithmic relation between carrier rate and time held good, but the value of the constant varied very widely, such figures as 0.018, 0.093, and 0.202 being obtained. Some adjustment is necessary because, in certain of these series, the test of clearance was a single negative swab, but the value of the constant in Hartley and Martin's series would only be altered to 0.032 on this basis, so that the rate of clearing under different conditions varies considerably—a fact which seems to merit further investigation.

A larger and equally illuminating series of figures recording the apparent rate of disappearance of diphtheria bacilli in convalescents at the North-Eastern Hospital was published by Thomson, Mann and Marriner (1928-29). Nose and throat swabs from proved cases of diphtheria were taken once during the 2nd and once during the 3rd week, and after that twice a week till six consecutive negative cultures had been obtained. Cases

TABLE 107
DISAPPEARANCE OF THE DIPHTHERIA BACILLUS FROM THE THROAT.

Days after Admission.	Number carrying (observed).	Number carrying (calculated).
5	392	—
10	302	310
15	232	242
20	194	189
25	156	147
30	118	115
35	92	89
40	70	70
45	62	64
50	41	42

in which the bacilli persisted for over 12 weeks from the beginning of the illness were regarded as carriers. Of 3,208 cases investigated, 242, or 7.5 per cent., became carriers. Table 108 shows the proportion of cases, exclusive of carriers, that became negative in successive weeks.

It will be seen that infection disappeared from the throat much quicker than from the nose. Analysis of these figures showed that when the throat only was affected the rate of clearance was practically the same at all ages, but that when the nose or nose and throat were affected the rate of clearance was slower in younger than in older patients. Of 285 carriers on which information is afforded, including the 242 already mentioned, 255, or 89.5 per cent., were infected in the nose or nose and throat, 26 in the throat only, and 4 in the ear only. The average time spent in hospital was roughly 7 months, and the longest 21 months. The age distribution of the total cases is not given, but it is mentioned that 55.7 per cent. of the carriers were under 7 years of age and only 4.5 per cent. over 13 years of age.

Wright (1941a), who records his experience at Liverpool on the rate of disappearance of diphtheria bacilli from the nose or throat of 311 convalescent patients, obtained results similar to those recorded by Hartley and Martin (1919-20). He made the interesting observation that clearance was most rapid in patients infected with the *intermedius*, and slowest in those infected with the *gravis* type. Examining swabs taken at weekly intervals and inoculated on to tellurite blood agar slopes, he found that a negative result was often

the Lancefield groups is, of course, a matter of detailed biochemical study the details of which may be found in Bergey (1948)

Pathogenicity for Animals. As indicated above, certain of the streptococci are responsible for specific diseases of domestic animals. *Str. equi* is the cause of strangles in horses, a suppurative infection of the upper respiratory tract that is characterized by abscess formation in the throat and submaxillary region. This species is apparently not pathogenic for man. Other strains of Group C infect horses, causing respiratory catarrh and suppurative lesions in various parts of the body. The most common cause of streptococcal mastitis in the cow is *Str. agalactiae* which produces a chronic infection that is, as a rule, more common in older cattle. The infection is probably spread by the hands of the milker for the most part. Mastitis may also be caused by *Str. pyogenes* and may result in the spread of milk borne streptococcal infection of man; it is probable that the source of infection is man and that *Str. pyogenes* does not occur naturally in the cow. Various other suppurative conditions, often involving infection of lymphatic tissue in animals such as the dog, sheep, etc., are of streptococcal etiology. Usually these are β hemolytic streptococci of groups other than Group A, but there are a few reports of spontaneous infections of lower animals with green streptococci.

Str. pyogenes is pathogenic for most laboratory animals, including the mouse, guinea pig and rabbit, but different strains vary widely in virulence for different animals, virulence may, of course, be enhanced by animal passage. Intravenous inoculation of virulent strains results in a fatal septicemia, suppurative peritonitis developing into septicemia follows intraperitoneal inoculation, and subcutaneous inoculation produces an abscess from which the infection may or may not spread. *Str. agalactiae* is of low virulence for experimental animals, and *Str. equi* is highly virulent only for the mouse. The green streptococci are also relatively avirulent for laboratory animals but may produce local infections on intravenous inoculation following injury; such techniques have been used to produce experimental arthritis lesions in the rabbit.

Pathogenicity for Man. The streptococci are responsible for a wide variety of diseases of man, perhaps a greater variety than any other kind of bacteria and, in addition to being the primary cause of disease, they have a marked tendency to occur in mixed and secondary infections with other pathogenic bacteria. In general the streptococcal infections are characterized by suppurative lesions and very often manifestations of toxemia, the latter taking the form of the so called non-suppurative complications of the infection and including fever, arthritis, carditis and nephritis. They vary in the extent to which the body is involved, from local infections such as abscesses of the various tissues, including mucous membranes, joints and serous membranes, infection of the muscle or cellulitis which simulates gaseous gangrene, suppurative processes in all kinds of wounds, etc., to those generalizing to pyemia or septicemia. Some streptococcal infections have no distinctive clinical manifestations, thus an abscess caused by streptococci is not distinguishable from one of staphylococcal etiology. Others, however, such as erysipelas, streptococcus sore throat, scarlet fever, and the like have to a greater or lesser degree some distinctive clinical character.

The β hemolytic streptococci are by far the most virulent and, as indi-

predominantly *gravis* type by a *mitis* (Report 1949); this supports the conclusion reached on other grounds that the *mitis* type is better able than the more toxic organisms to establish a commensal relationship with the host (see Leete 1945) (For general study of epidemiology of diphtheria, see W. T. Russell 1943)

The Diagnosis of Infection in Diphtheria.

The laboratory diagnosis of infection with diphtheria depends on the demonstration of the causative organism. Though we are concerned here more with general principles than with technical details, it will be convenient to describe the current practice in most British laboratories. In the past chief reliance was placed on examination of a direct smear, and on the microscopic appearance of the growth on a Loeffler slope culture. Since the differentiation by McLeod and his colleagues (Anderson *et al.* 1931, 1933*a, b*) of three different colonial types of diphtheria bacilli on tellurite blood agar, and since the agreement reached by numerous workers on the advantage of using a selective medium, the study of the colonies developing on tellurite plates has assumed increasing importance in the laboratory diagnosis of diphtheria. Not only does the isolation of diphtheria bacilli in the form of single colonies carry greater assurance than their demonstration morphologically in a film containing numerous other organisms, but the recognition of their type—*gravis*, *intermedius*, or *mitis*—proves often of epidemiological and sometimes of clinical interest. We may outline the general procedure to be followed and the interpretation of the results in looking for the diphtheria bacillus in suspected cases of the disease, and in convalescents, contacts, and suspected carriers.

Suspected Cases of Clinical Diphtheria.

By examination of a *smear* made directly from the swab and stained preferably by the wet-film technique, it is sometimes possible to make a presumptive diagnosis of diphtheria. Though this method is advocated by a few workers, the majority of bacteriologists do not regard it with favour. It is restricted in its usefulness to throat swabs from children, and even in these the proportion of positive results in true cases of diphtheria is often fairly low. Though it should not do so, a negative result serves as an excuse to many practitioners to withhold serum for treatment. A preliminary negative report on a direct smear has frequently to be followed by a positive report on culture, thus confusing the practitioner and leading him to doubt the accuracy of the laboratory's findings. Moreover, the time taken in preparing and examining direct smears may often be spent more profitably in other ways. Without denying the value of the method in special cases, most bacteriologists are agreed that for routine work it is much better abandoned. In some laboratories a film is made from throat swabs and stained for the characteristic spirilla and fusiform bacilli of Vincent's angina. If these organisms are found microscopically in the cultural absence of diphtheria bacilli and of haemolytic streptococci, they may afford an explanation of the clinical condition of the patient, and may be reported without prejudicing his treatment.

In the routine cultivation of swabs from suspected cases of diphtheria it is advisable to inoculate each swab on to a slope of Loeffler's serum, a plate of tellurite blood agar, and, for the detection of haemolytic streptococci, on to an ordinary blood agar plate. All three cultures should be examined 12–15 hours later. Positive results obtained on both Loeffler and tellurite, or on tellurite alone, can be

cated above, the great majority of those infecting man are of Group A, *Str. pyogenes*. The small proportion of infections with Group C streptococci are not distinguishable from those of Group A except by isolation and immunological typing of the etiologic agent. The pathogenicity of these bacteria is accounted for to a considerable degree by the soluble toxic substances they produce. The most clear-cut example is that of the relation of the erythrogenic toxin to scarlet fever, but it is highly probable that other toxic substances also play a part in the development of the pathologic condition.

It seems plausible that the pronounced invasive tendencies of the streptococci are due in part to the elaboration of hyaluronidase by those varieties that are not encapsulated with hyaluronic acid. The association of fibrinolysin with virulence seems definite, so much so that this activity has been termed invasins by some workers. The role of the streptolysins in the development of the pathology of streptococcus infections is not too clear. Both streptolysin S and streptolysin O are toxic for experimental animals, the former causing death through intravascular hemolysis. The mechanism of the lethal action of streptolysin O is not known; it may possibly be related to the cardio-toxic action of this substance described by Bernheimer. There is some reason to believe that the destructive effect of streptococcal filtrates on polymorphonuclear leucocytes, attributed to the presence of a leucocidin, is closely related to or identical with streptolysin O, but in any case this activity may make up a significant part of the invasive and pathogenic properties of the streptococci. While the streptococci contain no endotoxin in the usual sense, it is of interest that the presence of the type-specific "M" antigen is associated with virulence, and antibody to it is protective while antibody to the type-specific "T" antigen is not.

In general, then, while knowledge of the mechanisms of the pathogenic action of the β hemolytic streptococci is far from complete, there is a considerable body of evidence which indicates that the production of disease is due at least in part to the action of the several toxic substances formed by these bacteria. As indicated earlier, strains of streptococci differ with respect to the kinds and amounts of such substances that they produce, and this variability accounts in part for the differences in disease they may produce. Thus, both strains which produce erythrogenic toxin and those which do not may produce septic sore throat, but only the former can produce scarlet fever also. These are not the only factors, of course, and the route of infection and immunity of the host are significant also; for example, wound infection and streptococcal sore throat involve different routes of infection, and an erythrogenic toxin-producing strain can produce sore throat but not scarlet fever in the immune.

Streptococci other than the β hemolytic varieties are much less virulent. The α hemolytic forms constitute, as pointed out elsewhere, a portion of the normal bacterial flora of the mouth, upper respiratory tract and intestinal tract. It is probable that they seldom initiate infection of the healthy tissues, but when natural resistance has been reduced they may be able to set up low grade, essentially localized infections such as focal abscesses in the teeth and gums. They are the most common cause of subacute bacterial endocarditis but, while the condition is a serious one, the infection shows little

Loeffler slope and a tellurite plate and the growth examined the following day (see Brahdý *et al.* 1935). Occasionally strains of diphtheria bacilli are met with that are unusually sensitive to potassium tellurite, and may not develop on primary culture in the presence of this substance; to avoid missing these, a Loeffler serum culture is a valuable safeguard. On the other hand, the presence of apparently typical diphtheria bacilli on Loeffler in the complete absence of diphtheria colonies on tellurite must always be regarded with suspicion, and a final positive report should never be sent till diphtheria bacilli have been isolated in pure culture by plating the Loeffler slope growth on to chocolate blood agar or tellurite blood agar.

The routine use of a tellurite blood agar plate is of value partly because it increases the proportion of positive results, and partly because it enables the type of the infecting organism to be determined. By careful examination of the colonial form under a binocular dissecting microscope and of the morphology of the organisms in a wet-stained film, or even by nigrosin (Fleming 1941), many workers of experience can assign correctly the majority of cultures to the *gravis*, *intermedius*, or *mitis* types; but it is always wise to confirm this preliminary identification by means of fermentation and, if necessary, virulence tests.

Shone, Tucker, Glass, and Wright (1939), who made a very careful study at Liverpool of the comparative merits of Loeffler and tellurite in the diagnosis of diphtheria cases, found that of 1,501 strains of diphtheria bacilli 92.4 per cent. were recognized on both a Loeffler slope and a tellurite blood agar plate, 2.3 per cent. on Loeffler only, and 5.3 per cent. on tellurite only. The failures of the Loeffler medium seemed to be due partly to the scantiness of the diphtheria bacilli in some cases and the consequent difficulty of finding them among other bacteria, and partly to the atypical appearance of certain strains, especially those of the *gravis* type. The chief disadvantage of the tellurite medium lay in the slow growth of some strains of the *mitis* type. Cooper and his colleagues (1940), who made a similar series of observations in McLeod's laboratory at Leeds, found that a tellurite blood agar plate yielded at least 10 per cent. more positive results than a Loeffler slope, and led to less likelihood of missing severe cases. On Loeffler the very short form of *intermedius* type of diphtheria bacillus was found to be difficult to distinguish from unusually granular Hofmann strains. The results on Loeffler's medium at Liverpool were more favourable than those obtained in many other laboratories, probably because Wright and his colleagues had spent two years' intensive work checking the accuracy of their microscopical diagnosis. In the hands of less skilled observers the proportion of true positive results on Loeffler would have been lower, and a number of false positives would almost certainly have been recorded (see Cruickshank 1943).

Convalescents, Contacts, and Carriers.

In swabs from convalescents, contacts, and carriers diphtheria bacilli are generally fewer than in those from cases. The use of tellurite medium in their detection is not only an advantage; it is almost imperative. Numerous workers have found that approximately twice as many positive results are obtained on this medium as on Loeffler's serum. The development of the colonies is, however, often slow, and a negative report should rarely be made till the plates have been incubated for 48 hours. In contacts diphtheria bacilli may sometimes be isolated during the incubation period (Wildfuhr 1949).

or no tendency to spread throughout the body in spite of the frequent presence of the streptococci in the blood stream. Similarly, the α hemolytic streptococci are associated, probably causally, with rheumatic fever and arthritis, but the lesions are local and hypersensitivity to the streptococcal cell substance may be of considerable importance in the development of the disease. Of the anhemolytic streptococci, most are harmless saprophytes found in milk and dairy products, and these forms have been definitely associated only with subacute bacterial endocarditis, and then in a very small proportion of cases.

Epidemiology of Streptococcal Disease. The primary source of pathogenic streptococci is the human being who carries these bacteria in the upper respiratory tract. The infection may not be associated with symptoms and Group A and β hemolytic streptococcus carrier rates of 4 to 25 per cent have been reported by various workers. Those with overt symptoms of disease such as tonsillitis, pharyngitis, sinusitis and scarlet fever are, of course, prolific sources of infection. The importance of the carrier in the dissemination of streptococci has been studied extensively by Hamburger and his co-workers²³ who have shown that, while streptococci may be present in the saliva as well as in the throat, and discharged by sneezing, coughing and contamination of the hands, the nasal carrier is by far the most dangerous and contributes very large numbers of streptococci to his environment. Not only is the carrier the source of infection, but the fact that streptococcal disease may take a variety of clinical forms must be borne in mind. In an epidemic of scarlet fever, for example, the cases of pharyngitis and rhinitis are quite as important as those of frank scarlet fever, i.e., those showing a rash, in the spread of the infection, in fact many workers record the incidence of scarlatinal rash in a given epidemic rather than differentiate scarlet fever from other streptococcal infection of the upper respiratory tract.

The transmission of streptococci from the infected person to the susceptible individual is in part a matter of direct contact and in part one of contamination of the environment, as Loosh and his co-workers²⁴ have pointed out. Direct contact may include inhalation of infective droplets expelled from the nose and mouth, hand to hand contact, etc., while contamination of the environment is the contamination of the air with droplets too small to settle, and through air and droplet infection the contamination of dust. Direct contact with the hands no doubt accounts for wound infection, puerperal fever, infection of the udder with *Str. pyogenes* to produce mastitis and milk-borne streptococcal disease, and possibly infection of the upper respiratory tract to a certain extent. Most upper respiratory tract infection is air-borne, either directly or through the agency of resuspended infected dust. The importance of the last is very great indeed (p. 231), and dust suppression measures such as oiling of blankets in hospital wards sharply reduce the incidence of streptococcal infection.

Immunity to Streptococcus Infection. Antibodies to the streptococcus cell substance and to the antigenic soluble products of these organisms are

²³ Hamburger *et al.* Jour. Inf. Dis., 1944, 75:58, 71, 79, *ibid.*, 1945, 77:68, 96, Jour. Amer. Med. Assn., 1946, 130:836, Jour. Inf. Dis., 1946, 79:33.

²⁴ Loosh *et al.* Jour. Inf. Dis., 1948, 82:59, 72.

of filter paper impregnated with diphtheria antitoxin containing 1,000 units per ml is implanted in an agar plate while the medium is still fluid. When the agar has set, the organism to be tested is streaked at right angles to the strip. If toxin is produced, it precipitates the antitoxin in the form of double arrow-headed lines, the exact configuration and thickness of which depend on the occurrence of optimal proportions of toxin and antitoxin in different parts of the plate. This test has the advantage of rapidity, since with most strains the results can be read within 24 hours. Good agreement was reported between the *in vitro* and *in vivo* methods (Ouchterlony 1949, King *et al.* 1949).

The Diagnosis of Susceptibility or Immunity to Diphtheria.

Modern methods of diphtheria control depend almost as much on distinguishing between the susceptible and resistant members of the human herd as on diagnosing cases and carriers. The technique that first enabled us to do this on an adequate scale was the test introduced by Schick (Michiels and Schick 1913a, b, Schick 1913). It is performed as follows.

The test toxin is stored in a relatively concentrated solution, and when required for use is diluted so that one Schick dose is contained in 0.2 ml. Part of this diluted toxin is heated to 70° C. for 5 minutes, to serve as a control. Into the flexor surface of one arm 0.2 ml. of the unheated toxin is injected intradermally, care being taken that the injection is made into the substance of the dermis in such a way as to raise a definite bulla. Into the flexor surface of the opposite arm is injected 0.2 ml. of the heated toxin. The reaction to the toxin develops at a varying rate. In some persons a positive reaction is evident after 24 hours, but in others it is not fully developed for a week. From the observations of Downie and his colleagues (Report 1942), the best times to make readings would appear to be the 4th and the 7th days; if only one reading can be made, the 7th day should be selected. The following types of reaction may be observed to the toxin and the control:—

- (a) The negative reaction—no reaction of any kind in either arm.
- (b) The positive reaction—no reaction in the control arm.

In the test arm a circumscribed red flush appears generally after 24 to 36 hours, reaching its maximum development on the 4th to 7th day. At this time there is a circular area, 1–2 cm. in diameter, slightly raised above the general surface of the skin. This slowly fades during the following few days or more, leaving an area of brownish pigmentation, with a desquamating epidermis.

- (c) The pseudo-reaction (negative).—This reaction, which is of the characteristic allergic type, develops equally in both arms during the first 24 hours. It is less sharply circumscribed than the true positive reaction, and fades much more rapidly. By the 4th day it has usually disappeared, leaving some slight degree of reddish or brownish discoloration.

- (d) The combined reaction (pseudo + positive).

The control arm shows the succession of changes referred to under the pseudo-reaction. The reaction in the test arm is almost indistinguishable from that in the control arm during the first 24 hours. After this time the reaction in the test arm continues to develop, while that in the control arm commences to fade. By the 4th day the difference between the two arms is usually quite distinctive.

The positive and combined reactions are generally regarded as indicative of susceptibility. More precisely they show that antitoxin is either absent from the circulating blood or is present in only very small quantity. At one time it was taught that a positive reaction showed that there was less, and a negative reaction that there was more, than 1/30 unit of antitoxin per ml., but further experience has rendered it clear that either reaction may occur over a fairly wide range of antitoxin values (see Jensen 1931, Fraser and Halpern 1935, Leach and Póch 1935, Parish and Wright 1938, Thelander 1940, Downie

formed following immunization or infection. In the case of *Str. pyogenes*, the former include the type-specific "M" and "T" antigens as well as the group-specific "C" substance. In the latter group are the erythrogenic toxin, streptolysins S and O, hyaluronidase and fibrinolysin. Antibody formation has no diagnostic utility in acute streptococcus infections, in part because there is not sufficient time for antibody formation during the course of the disease, at least in its early stages, and in part because isolation, and typing if desirable, of the infecting microorganism are relatively simple. Antibody titration is useful, however, for diagnosis in retrospect, so to speak, in associating streptococci with diseases such as rheumatic fever and arthritis, and for the determination of susceptibility to scarlet fever on the basis of antibody to the erythrogenic toxin.

The immunized animal responds to the cell antigens of streptococci with the production of precipitating and agglutinating antibody, and the use of such antisera makes possible the immunological grouping and typing of these bacteria discussed earlier. The immune response of man to these antigens during the course of infection appears more often to take the form of the development of a hypersensitivity, especially in the rheumatic diseases, and the immunological response may be measured by intradermal inoculation of soluble streptococcal antigens.²⁷ More often, however, serum is titrated for antibody to streptolysin or fibrolysin by *in vitro* methods. Quite aside from the question of the etiology of such diseases, it is clear that the occurrence of such antibodies in the human population is not uncommon, as might well be expected from the frequency with which streptococcal infection occurs, and it is clear that such infections do give rise to an antibody response. The erythrogenic toxin also stimulates the formation of specific antitoxin, following both clinical scarlet fever and immunization with the toxin, and, like diphtheria antitoxin, the incidence of its occurrence rises with age, indicating that immunization occurs without the intervention of clinical scarlet fever. Intradermal inoculation of erythrogenic toxin gives a skin test, analogous to the Schick test in diphtheria, which allows the estimation of the immunity of the individual to the toxin. This test, the Dick test, is discussed in the later section on scarlet fever. Antibody to the erythrogenic toxin cannot be titrated by *in vitro* methods, or to a satisfactory degree in experimental animals because of their relative lack of susceptibility to its action.

Differentiation must be made between an immune response in the technical sense of antibody formation and an effective immunity which prevents and/or modifies the infection or disease. The only immunity to streptococcal disease that is unquestionably effective is immunity to the erythrogenic toxin, which is reflected as immunity to the clinical disease scarlet fever. In general, however, immunity to streptococcus infections is of a low order and in any case transient. That some degree of effective immunity to a given type of streptococcus may be produced is indicated by recovery from and elimination of the infection in naturally occurring disease, and there is also some experimental evidence of the existence of an effective immunity. Wat-

²⁷ See, for example, Taran, Jablon and Weyr. *Jour Immunol.*, 1944, 49 209, *ibid.*, 1945, 51:53.

immunization. The last method comprises both active immunization by means of an antigenic preparation now usually referred to as diphtheria prophylactic, and passive immunization conferred by the injection of antitoxic serum. Passive immunization, because of its transitory nature, has a very restricted application and will be dealt with later (p. 1589). For all ordinary field purposes active immunization is employed. An adequate understanding of the principles that govern the application of this method to the control of the disease is so important to the medical practitioner and the medical officer of health that the relevant findings must be set out in some detail. We shall begin by describing the various forms of diphtheria prophylactic and their standardization, proceed to a consideration of their application under varying conditions in practice, and then discuss some of the results that have followed their use in mass immunization.

The Various Forms of Diphtheria Prophylactic.

As early as 1892 von Behring and Wernicke showed that susceptible animals might be safely immunized by inoculating them with increasing doses of living culture after a protective dose of antitoxic serum. Six years later Nikanaroff reported successful results from the inoculation of a few doses of toxin neutralized with antitoxin; and Dreyer, in 1900, proposed the use of toxin-antitoxin mixtures during the early stages of antitoxin production in horses. For various reasons there was a long lag before similar methods were applied to man; and it was not till 1913, when von Behring reported the successful use of a toxin-antitoxin mixture for the immunization of children, that inoculation against diphtheria entered the field of prophylactic medicine. From then onwards the use of such mixtures for the active immunization of children developed very rapidly, particularly in the United States under the influence of Park and Zingher (Park 1913, 1918, 1922, Park and Zingher 1915, 1916, Zingher 1921, 1922). Toxin-antitoxin mixtures were found to be not without danger (see Park and Schroder 1932), and after the discovery by Glenny and Südmersen (1921), Glenny and Hopkins (1923), and Ramon (1923, 1928) that diphtheria toxin could be so altered by treatment with formalin as to deprive it of its toxicity without destroying its antigenic power, formol toxoid, or anatoxin as it is called in French-speaking countries, gradually began to replace the older mixtures. The further demonstration by Glenny (1930) that formol toxoid could be improved by precipitation with alum has now placed at our disposal an agent of high antigenic potency which, when properly prepared and used, can be relied upon to give a fairly high degree of protection against diphtheria. (For review of prophylactic agents, see Hartley 1949.)

Toxin-Antitoxin Mixture.—In preparations of this class sufficient antitoxin is added to neutralize the toxic effects of the toxin without completely destroying its antigenic activity. The toxin, that is to say, is under-neutralized. Though very satisfactory results have been obtained by the use of such mixtures, a few accidents have shown that the union of toxin and antitoxin is unstable and is liable to be disturbed by unsuitable methods of preparation or of storage. In one instance, in which 5 deaths and 40 serious reactions followed the use of this prophylactic (see Forbes 1927), it was found that, in forgetfulness of the Danysz phenomenon, the toxin had been added in fractions to obtain the correct toxin-antitoxin ratio. In another instance a particular phial of toxin-antitoxin mixture that had been frozen was found to have caused severe constitutional reactions, whereas other phials of the same batch that had not been frozen had been used without any untoward results. Further investigations revealed that the freezing of such mixtures frequently resulted in a considerable rise in toxicity. Pope (1927) has shown that this

son, Rothbard and Swift,²⁶ for instance, have shown that a nasopharyngeal carrier state induced in monkeys resulted in an increase in antistreptolysin O titer of the serum and a resistance to reimplantation with the same strain that persisted for some months. Unfortunately such immunity appears to be type-specific and, as pointed out earlier, associated with antibody to the "M" antigen, and there is only a small degree of cross immunity. With the multiplicity of streptococcus types in the species *Str. pyogenes*, an effective immunity to streptococcus infection appears to be a somewhat impractical end.

Bacteriological Diagnosis of Streptococcal Infection. The isolation of streptococci from specimens of pathological material is ordinarily not difficult. An enriched medium is required and blood agar is the medium of choice, for both α hemolysis and β hemolysis are apparent. Overgrowth by *Proteus* in cultures of some kinds of specimens may be prevented by including 0.02 per cent sodium azide in the medium. Most specimens, such as throat swabs, pus, etc., may be streaked directly on blood agar but enrichment culture, as in veal infusion broth containing 0.1 per cent dextrose and 0.1 per cent phosphate buffer, should be made with blood taken for culture, incubated for twenty-four hours, and then streaked on blood agar. If there is reason to believe that the specimen contains sulfa drug, its bacteriostatic effect may be neutralized by including 5 mg. per 100 ml of *p*-aminobenzoic acid in the medium.

The colonial morphology is typical in the case of β hemolytic streptococci and the characteristic chains of cocci may be found in gram-stained smears. Green streptococci from sputum and similar specimens must be differentiated from pneumococci by inulin fermentation and bile solubility. The hemolytic streptococci may be typed by agglutination with type-specific antisera and by the precipitin test. For the latter the sedimented bacteria from a 250 ml. broth culture are suspended in 10 ml. of N/10 HCl in saline, boiled for ten minutes, cooled in running water, and the insoluble material spun out to leave a clear supernatant to be used as the antigen. Considerable economy of reagents may be effected by setting up the precipitin test as a ring test in capillary pipettes, the precipitate at the serum-antigen interface may be observed with a hand lens.²⁷

STREPTOCOCCAL INFECTION OF THE SKIN AND SUBCUTANEOUS TISSUES

Erysipelas. The ability of streptococci to infect the skin and adjacent tissues is well illustrated in erysipelas, an inflammatory disease of the skin caused by *Str. pyogenes*. There is some evidence that attack of the disease is preceded by streptococcal infection of the throat or elsewhere in the upper respiratory tract, and it has been found that some individuals at least have the same immunological type of streptococcus in the throat as in the skin lesions. It is not clear whether the skin is directly invaded, or whether the microorganisms reach the area by some internal route, but the latter is only suggested and by no means established. The etiologic relationship of *Str. pyogenes* to the disease is indicated by its presence, frequently in enormous

²⁶ Watson, Rothbard and Swift. Jour. Exp. Med., 1946, 84:127.

²⁷ Swift, Wilson and Lancefield. Jour. Exp. Med., 1943, 78:127.

as A.D.F., consisting of toxoid-antitoxin floccules adsorbed on to aluminium phosphate. Tested in adults, it gave a good antitoxin response and caused fewer and less severe reactions than F.T. or A.P.T.

Active Immunization.

To produce a high degree of immunity against diphtheria by prophylactic inoculation, account must be taken of the nature of the prophylactic, the dosage, and the interval between the doses. Other factors, such as the latent immunizability of the population (Dudley, May, and O'Flynn 1934), and possibly the volume of the fluid in which the prophylactic agent is injected (Hartley 1935b), may influence the result, but need not be considered here.

Of chief importance is the *antigenic quality of the prophylactic*. As was pointed out in the last section, methods of standardization are still imperfect, and unless a high standard is set, batches of prophylactic may be issued that are unable to stimulate a satisfactory immune response. Even an increase in dosage as much as fivefold may fail to atone for a qualitatively poor antigen (Fulton *et al.* 1942).

Several careful studies have been made on the efficacy of different types of diphtheria prophylactic, as judged by the Schick-conversion rate or by the amount of antitoxin appearing in the blood serum (see, for example, Park and Zingher 1915, 1916, Park 1922, Ramon 1926, Kundratitz 1927, Parish and Okell 1928, Martin, Loiseau, and Laffaille 1928, Ramon and Debré 1931, Seligmann 1931, McGinnes, Stebbins, and Hart 1934, Hame 1935, Fraser and Halpern 1935, 1937, Jensen 1937, Faragó 1940; for references to results of two doses of A.P.T. Pösch and Schmid 1938, Chesney 1939, Fulton *et al.* 1941, Lewis 1941, Volk and Bunney 1939, 1942a, Freeman 1942; and of two doses of P.T.A.P. Bousfield *et al.* 1948, Mackenzie 1950). The findings may be summarized by saying that two properly spaced doses of A.P.T. or P.T.A.P. result in an average Schick-conversion rate of about 98 per cent., whereas with other prophylactics this high rate may be closely approached, but is seldom equalled.

The choice of prophylactic must be determined not only by its immunizing quality but also by the degree of local or constitutional reaction to which it gives rise on injection. In general, toxoids precipitated with alum or aluminium phosphate are more irritant than plain formol toxoid or toxoid-antitoxin mixtures. Reactions are commoner in persons giving a negative, a pseudo, or a combined response (see p. 1576) to the Schick test than in those responding to the toxin alone, and are seen more often in older children and adults than in infants or young children. It is wise, therefore, when immunizing subjects over 10 years old to use either one of the less irritant prophylactics, such as T.A.F., or, if an alum-precipitated toxoid is selected, to give it in small dosage. To judge the subject's sensitivity, the Schick test may be carried out beforehand. The response to this test, however, does not afford an infallible guide to the probable reaction to diphtheria prophylactic. For this reason the *Moloney* test (Moloney and Fraser 1927) was introduced. An injection of 0.2 ml. of a 1/20 dilution of formol toxoid is made intradermally. Any person giving more than a minimum local reaction should be immunized cautiously. This test likewise is by no means infallible and in practice is seldom used (see Boyd 1946).

The irritant effect of diphtheria prophylactic may be either of specific or of non-specific origin. Adult subjects tested with a purified, but by no means pure, toxoid fraction and with a fraction containing non-toxic diphtheria proteins reacted sometimes to one, sometimes to the other, sometimes to both, and sometimes to neither; reactions were much more common in Schick-negative than in Schick-positive subjects. Reactions to

also frequently persist in tonsillar crypts in a chronic type of infection which may flare up periodically in an acute form. Streptococcal tonsillitis, sinusitis and otitis media are, then, a part of the pathology of hemolytic streptococcus infection of the throat. In addition to such extensions, the effects of toxemia on other parts of the body are evident as carditis, nephritis and arthritic involvement of the joints. As in the case of scarlatinal rash noted earlier, the character of an epidemic is often recorded as percentage incidence of these various sequelae.

The distinctive clinical character and epidemic spread of the disease led earlier workers to believe that it was caused by a particular kind of streptococcus to which the name *Str. epidemicus* was given. It is now clear that various immunologic types of *Str. pyogenes* are, for the most part, responsible for the disease, and a small portion of the cases are infections with streptococci of Group C, now grouped as *Str. equisimilis*. On the other hand, there is reason to believe that so-called "epidemic strains," of high virulence and infectivity, of streptococci as well as other bacteria, are often associated with epidemic disease.

It is probable that the infection is largely droplet and air-borne, including dust, but there is no doubt that in many instances direct contact is of considerable significance. It may also be transmitted through the agency of food and milk, giving rise to epidemics among the consumers of these products. Hamburger, Green and Hamburger³⁰ have reported a food-borne epidemic of pharyngitis-tonsillitis among convalescent patients in which a food handler was found to be in the incubation period of pharyngitis-sinusitis caused by *Str. pyogenes* Type 1; his nose ran profusely, he had strongly positive nose and throat cultures, and 10,000,000 streptococci were recovered in a single culture of his hands. There is no doubt also that streptococcal infection is frequently milk-borne. In the past it has been assumed that the contamination was direct from man, but in recent years evidence has accumulated which indicates that mastitis of *Str. pyogenes* etiology may constitute the immediate source of infection of the milk.

SCARLET FEVER

Scarlet fever is a clinical entity because of the rash resulting from the action of the erythrogenic toxin, otherwise it does not differ significantly from other streptococcus infection of the upper respiratory tract and its sequelae are essentially the same. The relationship of β hemolytic streptococci to the disease was demonstrated by Dick and Dick in 1923³¹ by the reproduction of typical scarlet fever in human volunteers by the inoculation of pure cultures, and by the demonstration of the existence of the erythrogenic toxin. A conclusive demonstration of the etiologic relation was required because of the contrast between the relatively lasting immunity to scarlet fever following recovery from an attack of the disease and the transient immunity to other streptococcal infections.

It has been held by the Dicks and others, largely on the basis of early

³⁰ Hamburger, Green and Hamburger: *Jour. Inf. Dis.*, 1945, 77 96

³¹ For a general discussion see Dick and Dick *Scarlet Fever* Year Book Publishers, Chicago 1938.

discussion. Most authorities recommend 6-12 months, but partly in order to confer earlier protection against diphtheria and partly because of the convenience of combining diphtheria prophylactic with pertussis vaccine (see Bell 1948, Sant'Agnese 1950), there is a tendency to start immunization during the first 3 months of life. Though good results can be obtained at this age in individual subjects, particularly if three widely spaced doses of A.P.T. are given (Barr *et al.* 1950), the method is of doubtful value for purposes of mass immunization, because the passive immunity received from the mother has not always disappeared by the age of 3 months. Our own view is that, in this country in which routine inoculation is already established, it is wiser to postpone immunization till the end of the first year of life in order that the child's resistance may be greatest during the 2nd, 3rd and 4th years, when the risk of contracting the disease is highest. If the infant is immunized at 3-4 months of age, it will need a reinforcing injection at 18 months and again on entering school, instead of only one at 5 years of age (see Report 1953).

The duration of immunity is dependent not only upon the type of prophylactic, but upon the subsequent exposure of the inoculated subject to infection with the diphtheria bacillus. In a community in which there is much diphtheria or a high carrier rate the immunizing mechanism is likely to receive more or less frequent stimuli, which will have the effect of reinforcing the immunity conferred by the prophylactic. On the other hand, in communities with little or no experience of diphtheritic infection, the antitoxin content of inoculated subjects may be expected to fall progressively (see Jensen 1933*a*, *b*) and the Schick reaction to revert to positive. It is not surprising, therefore, that the results recorded by different workers are not in complete accord. Some observers, for example, like Park (1922), working in densely populated cities, have had few Schick relapses over a period of many years, whereas others, working in areas where there has been little diphtheria infection, have had high relapse rates. Thus Fraser and Brandon (1936), in Canada, found that of children who had received three doses of formol toxoid five years previously, and who had not been exposed to infection since, 31 per cent. had reverted to the Schick-positive state. Similar results are recorded by Schwartz and Janney (1938) and Volk and Bunney (1942*b*) in the United States, and by Sigurjónsson (1939) in Iceland (see also Parish and Okell 1928, Cooke 1936, Jones 1936-37, Christie 1940, Nervus and McGrath 1940, Duke and Stott 1913, Sellers *et al.* 1915). Generally speaking, we may say that in children properly immunized with two doses of A.P.T. and not exposed to diphtheritic infection the Schick-relapse rate should not exceed 5 per cent per year. Since immunity cannot be relied upon to last indefinitely, it is wise to reinoculate children every 4 or 5 years. For this purpose a single dose of prophylactic is sufficient, since there is abundant evidence to show that a single reinoculation of persons, even of those who have relapsed to the Schick-positive state, is followed by a rapid rise in the antitoxin content of the blood (see Parish and Okell 1928, Parish and Wright 1938, Fraser 1910, Volk and Bunney 1942*b*, Wishart *et al.* 1914).

It may be noted that many patients who have recovered from diphtheria remain Schick-positive (see Warin 1940). Since second attacks of the disease are not uncommon, such persons may be regarded as susceptible, and inoculated or re-inoculated with the rest. Why an attack of diphtheria is not always followed by immunity is still doubtful. That it is not due mainly to suppression of the antibody-forming mechanism by the administration of large quantities of antitoxin therapeutically seems clear from Madsen's (1933)

agglutination studies, that the scarlet fever streptococci constitute a homogeneous group of β hemolytic streptococci which should be designated *Str. scarlatinae*. While the streptococci found in scarlet fever are all members of Group A, i.e., *Str. pyogenes*, the ability to form erythrogenic toxin is not confined to any particular type within this group, though some types occur more frequently than others, and it cannot be said that the scarlet fever streptococci are immunologically homogeneous. Neither are they biochemically homogeneous, and in the Dicks' early experiments the mannitol fermentation was variable in scarlet fever-producing strains. It appears, then, that the scarlet fever streptococci are strains of *Str. pyogenes* that have in common

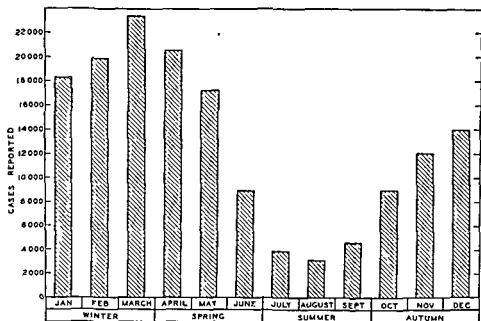


Fig. 49. The seasonal incidence of scarlet fever. Averages of reported cases by months for the years 1939 to 1945 inclusive. Data from Supplements to Public Health Reports.

the ability to form erythrogenic toxin, but such strains are found in conditions other than scarlet fever, such as erysipelas.

The Erythrogenic Toxin. The erythrogenic toxin differs from the other soluble bacterial toxins in that it is resistant to heat, as noted earlier. Its nature is not clear as yet, however. It has been reported to be a protein by some workers and a polysaccharide-containing complex by others.³² Barron, Dick and Lyman³³ have reported that it is a protein of low molecular weight and is resistant to proteolytic digestion and a number of oxidizing and reducing agents. Stock³⁴ has prepared a highly purified heat-coagulable protein containing 10^7 STD per milligram which he regards as identical with the erythrogenic toxin. Four components have been demonstrated by electrophoresis; the toxicity was associated with the slowest and on separation this contained 10^8 STD per milligram.³⁵ Whatever its nature, it is necessarily

³² Earlier work is reviewed by Eaton, *Bact. Rev.*, 1938, 2 3.

³³ Barron, Dick and Lyman, *Jour. Biol. Chem.*, 1941, 137 267.

³⁴ Stock, *Jour. Biol. Chem.*, 1942, 142 777.

³⁵ Krejci, Stock, Sanigar and Kraemer, *Jour. Biol. Chem.*, 1942, 142 785.

each group that are rendered Schick-negative. Unless a preliminary test is made, the proportion of original Schick-negative reactors in each group is unknown, and a difference in their distribution will affect the result of the comparison.

Complications directly attributable to inoculation against diphtheria are uncommon. There is some evidence that it may activate a latent poliomyelitis infection (McCloskey 1950, Hill and Knowelden 1950, Report 1951); and it may be wise therefore to suspend inoculation during the height of an epidemic of this disease. Aseptic meningitis has been described, but it must be a very rare sequel (Stullerman 1948). Sterile abscesses—or more correctly cysts—occur in a small proportion of persons inoculated with alum-precipitated products, but they generally disappear spontaneously without giving rise to more than local inconvenience.

The Protective Effect of Active Immunization.

Though a high Schick-negative or Schick-conversion rate affords a strong presumption that satisfactory immunization has been achieved, the ultimate court of reference in determining the efficacy of prophylactic inoculation must be the behaviour of immunized persons when exposed to the risk of natural infection.

Bieber (1920) reported the after-history, from 1913, of 1,097 immunized and 3,275 non-immunized children in villages near Magdeburg. Of the former, 52 (4.8 per cent) had contracted diphtheria; of the latter, 493 (15.1 per cent.). Park (1922) records the history over a shorter period of 90,000 immunized or naturally immune children and 90,000 controls. During the period of observation 14 of the former contracted diphtheria as against 56 of the latter. Adams (see Fitzgerald 1928) records the history during the years 1926 and 1927 of 11,000 children who had received two immunizing doses of toxoid, and of 9,000 non-immunized controls. Among the immunized the incidence of diphtheria was 1.55 per 1,000, among the non-immunized it was 11.44 per 1,000. Isabolowski and others (1931) describe the results of immunization in Smolensk. Of 4,185 children immunized with toxoid, 0.14 per cent. contracted diphtheria during the eleven months following immunization; of 19,000 control children in the same town 1.5 per cent. contracted diphtheria during the same period. McKinnon and others (1931) and Fitzgerald and others (1932) record the results of immunization in Toronto. Among a group of 36,189 children who had passed through the immunization centres and had been found to be Schick-negative, or had received one or more immunizing doses of toxoid, or had been found to be sensitive to a preliminary injection of toxoid and so received no further treatment, there were 120 cases of diphtheria during the period of observation (December 1926–June 1929). As a control the prevailing rates of diphtheria morbidity at ages among the non-immunized population were applied to each age group of the immunized. This gave 478 as the expected number of cases. Of the 36,189 children, 16,829 had received three doses of toxoid. Among this group 222 cases of diphtheria would have been expected; only 23 occurred. There are many other records of an analogous kind (see Forbes 1932). More recently, in Scotland, there were 2,833 cases of diphtheria and 13 deaths among about 750,000 immunized, and 17,091 cases and 794 deaths among rather less than half the number of non-immunized persons. The risk of contracting diphtheria was about 14 times less in the immunized than in the non-immunized, and the risk of contracting fatal diphtheria was over 100 times less (A. Russell 1943).

In England and Wales 4,829,115 children under 15 years of age were immunized between 1940 and 1943. The estimated child population under 15 years in 1943 was 8,527,000. Analysis of the 1943 returns showed the following rates for diphtheria:

Annual rates of incidence per 1,000 child-years:

(a) In immunized children	1.16	} $n : b = 1 : 3.5$
(b) In non-immunized children	4.06	

assayed by skin test and its potency is measured in skin test doses (STD), the smallest amount that will produce the characteristic erythematous response. It is, of course, antigenic, and is flocculated by antitoxic sera.

Scarlet Fever Antitoxin. The therapeutic use of antistreptococcus sera was investigated by Moser in 1902 with encouraging results, and similar observations were reported by Dochez and others in 1924. The Dicks prepared specific antitoxic sera by immunizing horses with sterile culture filtrates. The results of the therapeutic use of the Dick antitoxin are favorable on the whole, though the mildness of the prevalent type of scarlet fever makes it difficult to secure any large body of statistics as cogent as those recorded in diphtheria. In individual cases, however, the administration of antitoxin decreases the duration of the rash, changes the character and extent of desquamation and reduces the number of complications, and there is general agreement as to the efficacy of antitoxic serum properly prepared and administered early. Some clinicians would restrict the use of scarlet fever antitoxin to cases of severe or toxemic type. A unit of antitoxin is defined as that amount which will neutralize 50 skin test doses of toxin.

Immunity to scarlet fever is, in a sense, a clinical immunity in that it is largely an immunity to the erythrogenic toxin rather than to the streptococcus. It may be demonstrated by the *Dick test*, a skin test analogous to the Schick test in diphtheria, *i.e.*, the local erythema is due to the action of the toxin and is absent in the presence of antitoxin, either of exogenous origin or present in the immune individual. The Dick test may be used, then, to ascertain whether or not an individual is immune to scarlet fever or, more precisely, whether circulating antitoxin is present. In this connection it is of interest that Schulz and Charlton³⁶ earlier observed that when a scarlet fever patient with a bright-red rash is injected with 1 ml. of convalescent serum, after about six hours the rash begins to fade and soon disappears completely. The significance of this phenomenon, the *Schultz-Charlton blanching phenomenon*, was not recognized at the time.

Prophylactic Inoculation. It is not generally known that following Jenner's work on smallpox vaccination (p. 6) attempts were made to immunize against scarlet fever by a similar process of inoculation. Preventive inoculation with killed streptococcus cultures was practiced by Russian bacteriologists as early as 1906. Mild symptoms were produced similar to those observed in scarlet fever. A single injection did not suffice to produce immunity, two or three inoculations being necessary. It was believed that a considerable degree of immunity was obtained by this procedure.

The discovery of the scarlet fever toxin offered an opportunity for protective immunization similar to that successfully utilized in diphtheria. Toxins with a potency of at least 40,000 STD per cubic centimeter are preferable, and appropriate dilutions are injected at intervals of one week. Five injections are recommended, starting with 500 STD and gradually rising to about 100,000 STD. Immunization of susceptibles (Dick positives) in this manner produces 98 per cent or more Dick negatives. The injections are sometimes accompanied by the development of a scarlatiniform rash and other symptoms of mild scarlet fever. In consequence, the use of toxin

³⁶ Schultz and Charlton: *Ztschr. f. Kinderheilk.*, 1918, *Orig* 17:328.

of immunity is raised above a critical level—the level in any given community depending on many different factors—the chain of infection is broken and the disease tends to die out. To achieve this end it would appear that in a fairly densely populated urban community at least 70 per cent. of school and pre-school children must be immunized and kept immune by reinforcing injections. Even this may prove insufficient if other factors are unfavourable, such as, for example, invasion with a particularly virulent strain of *gravis* type.

Objections have been raised to diphtheria inoculation on the ground that it may lead to an increase in the proportion of healthy carriers and so perpetuate or even aggravate the disease. We are not thinking here of what happens in an institution, but in the general population. Clearly if diphtheria is introduced into an

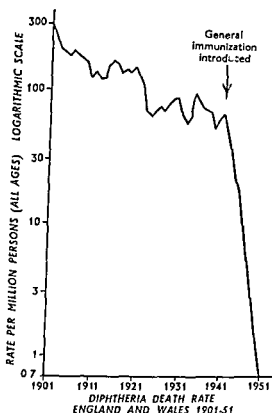


FIG. 284.

Showing the precipitous fall in the death rate from diphtheria in England and Wales since the introduction of general immunization. (From Logan 1952.)

institution containing a high proportion of Schick-negative reactors, there is bound to be a temporary rise in the carrier rate, since the majority of those who become infected will not develop clinical diphtheria and will therefore not be removed to an isolation hospital. In the general population, however, one would expect a different sequence. The fewer cases there are as the result of immunization, the fewer contact carriers will there be, and the fewer carriers there are, the less will be the chance of non-immunes developing diphtheria. On the whole, this assumption is borne out by American experience, where in some towns the carrier rate among the general population has fallen to almost negligible proportions after continued mass immunization. The few figures available in this country are in conformity with the findings in America. Thus, before the second world war the carrier rate in London elementary school children varied between about 2.5 and 5.0

detoxified by treatment with formalin has been advocated by some workers. Veldee³⁷ has reported that a purified precipitated toxin apparently produces a good immunity with fewer untoward reactions. It is to be emphasized, however, that immunity to scarlet fever is an immunity to clinical scarlet fever, not to the streptococcal infection, and from the epidemiological point of view both Dick-negative and Dick-positive individuals must be considered as having scarlatinal infections, the only difference being the clinical one dependent upon the development of a rash.

As in the case of diphtheria, immunity to scarlet fever may be acquired by inapparent infection (p. 621). The frequency of positive Dick tests is low in newborn children (indicating a passive immunity of maternal origin), then rises to a maximum in the age groups one to five years, and thereafter

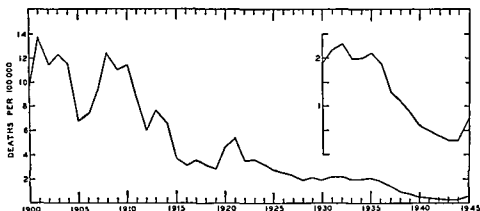


Fig. 50. The prevalence of scarlet fever in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

falls off gradually until in persons over thirty it is relatively low, possibly 15 per cent.

RHEUMATIC FEVER

As indicated earlier, not uncommon sequelae of β hemolytic streptococcus infection include carditis and arthritic involvement of the joints. These are emphasized and assume a major role in the pathology and symptomatology of the post-streptococcal non suppurative inflammatory disease known as rheumatic fever, acute rheumatism or rheumatic heart disease. The essential lesion of rheumatic fever is carditis which may or may not be accompanied by pyrexia and arthritis. The carditis includes the connective tissue degeneration characteristic of the damaged heart valves, and specific inflammatory myocardial lesions characterized histologically by nodular collections of cells described by Aschoff and known as Aschoff nodules.³⁸

The connection between β hemolytic streptococcus infection and rheumatic fever is very close and there is good reason to believe, though as yet not complete and unequivocal proof, that the relationship is etiological. The epi-

³⁷ Cf. Veldee, Peck, Franklin and DuPay. Pub. Health Repts., 1941, 56 957.

³⁸ See the general reviews by Griffith: Jour. Amer. Med. Assn., 1947, 133 974, and Lancet, 1947, i.217.

by the appearance of diphtheria cases due to infection from healthy carriers. The combination of active and passive immunization results not only in a transitory protection of the exposed children, but in the development of an active immunity that can generally be relied upon to prevent further cases of diphtheria developing, even in the presence of continued infection. Since, however, there is an intermediate period of relative susceptibility between the wearing off of passive and the development of active immunity, it is wise to swab the entire population and segregate all virulent carriers till a fortnight after the second dose of A.P.T. By this time sufficient active immunity will generally have developed to render almost negligible the risk of further cases occurring. By this method very successful results were reported by Fulton, Taylor, Wells, and Wilson (1941) in the control of school and institutional epidemics. To the paper by these authors and to two papers by Downie and his colleagues (1941, 1948) reference may be made for the rationale and application of combined active and passive immunization.

The advantage of this method in conjunction with swabbing of the entire population and segregation of virulent carriers is that school closure is quite unnecessary, and that, apart from the carriers who are segregated for 6 weeks, every child can continue its normal education. Little is to be gained by preliminary Schick-testing of the population, as was recommended by Okell, Eagleton, and O'Brien (1924) in the days before combined active and passive immunization was shown to be practicable, and valuable time may be lost. Moreover, the danger of diphtheria developing in Schick-negative reactors who are exposed to special risk of infection is by no means remote. On the whole, we think that there is far more to be gained by giving combined active and passive immunization to all children than by immunizing only the Schick-positive reactors. The time and labour expended are also less.

The Treatment of Diphtheria with Antitoxin.

The nature of antitoxic immunity and the general principles that determine its practical application have been discussed in Chapter 46. We are here concerned with the methods that are employed in the particular case of diphtheria in man, and the available information on their efficacy.

Antitoxic serum is prepared commercially by the injection of horses with increasing doses of toxoid. In practice, only animals containing natural diphtheria antitoxin in their blood are chosen, since they respond so much better than others to immunization. Injections are made intramuscularly two, or three times a week till a dose of half a litre or more can be given. When the level of antitoxin has risen high enough, three bleedings of 8 litres each are taken over a period of eight days. The horse is rested for a week or two and then given a short course of five injections, after which it is bled again. The process may be repeated four or five times. The serum is separated from the clot, filtered, and preserved with an antiseptic such as 0.3 per cent. tricresol (Dean 1908). Serum obtained in this way may be expected to contain about 1000 units per ml. of antitoxin.

Apart from its great theoretical interest, the isolation of antitoxin in a state of chemical purity would be an important advance from the practical point of view. This has not yet been achieved, but considerable success has been attained in the concentration of the active constituents (Brieger and Ehrlich 1893, Brieger and Cohn 1893, Brieger and Boer 1896, Pick 1902, Gibson 1905, Banzhaf and Gibson 1907, 1908-9, Banzhaf 1912-13, Andrewes *et al.* 1923). The methods employed, which vary in minor points of technique,

PUERPERAL FEVER

Puerperal fever is a vague term in that some degree of pyrexia is not uncommon immediately following childbirth. A definite febrile response is probably in most instances associated with bacterial infection but in mild cases the microorganisms are relatively avirulent. The severe infections, however, are due almost entirely to streptococci, the majority *Str. pyogenes*. Infection with other β hemolytic streptococci occurs but when these are of groups other than Group A the infections are usually not severe. The anaerobic streptococci are second only to *Str. pyogenes* in importance, being the cause of possibly 20 to 25 per cent of cases of severe puerperal fever; the disease caused by these forms is not as rapidly fulminating as that caused by *Str. pyogenes*, but the case fatality rate is high, possibly 40 per cent. In fatal cases of puerperal fever the infection generalizes to a fatal septicemia and it is highly probable that the pronounced invasive qualities of the streptococci are responsible for their virulence under these circumstances.

A point that has been of primary interest is the source of the streptococci producing puerperal fever. It seems well established that *Str. pyogenes* occurs only rarely in the female genital tract and is rarely found before labor or during an afebrile puerperium. Lancefield and Hare,⁴⁵ for example, reported a series of 855 cultures of the vagina of parturient women; one strain of *Str. pyogenes* was found among 65 strains isolated during an afebrile puerperium, and none among 13 strains isolated before labor. This and similar studies indicate that the source of infection with *Str. pyogenes* is exogenous rather than endogenous. The development of streptococcus typing and a reasonably precise definition of the immunological types has allowed the demonstration of the probable source of the infecting organisms. In the studies reported by Smith⁴⁶ slightly over 50 per cent of the streptococci from the patient were identical with those from the nose and throat of attendants, and a little less than 25 per cent identical with those from the nose and throat of the patient. Colebrook⁴⁷ reported similar results, the percentages being 58 and 38 respectively. Data such as these show clearly that the source of infection is exogenous with respect to the genital tract and most probably the nose and throat of attending persons or the patient herself in 75 per cent or more of cases. The infection may be air- or dust-borne, but it seems probable that the hands play a significant part in the transfer of infection.

The anaerobic streptococci, on the other hand, are normal inhabitants of the human vagina. In the absence of precise immunological methods for their identification, it may be tentatively concluded that infection with these organisms to produce puerperal fever is probably endogenous for the most part.

⁴⁵ Lancefield and Hare: Jour. Exp. Med., 1935, 61.335.

⁴⁶ Smith: Causation and Source of Infection in Puerperal Fever. H.M. Stationery Office, London, 1931

⁴⁷ Colebrook: Med. Res. Council, Spec. Rept. Series No. 205, 1935.

DIPHTHERIA AND OTHER DISEASES DUE TO CORYNEBACTERIA

advanced stage, though every effort should be made to ensure that it is given early in the disease.

A system of dosage in common use is that advocated by Park (1921) (see Table 110); but there has been a tendency to increase the amount of antitoxin given in the severer type of case as the result of the experience of recent years, and this tendency seems likely to continue. Thus, Bie (1922), in an admirable review of the evidence in regard to the value of antitoxin treatment in diphtheria, advocates the administration of very large total doses (92,000—170,000 units). Harries (1945) likewise recommends a large single dose, but considers that it should rarely be necessary to give more than 50,000 units, provided part or the whole of the dose is given intravenously.

TABLE 110

UNITS OF ANTITOXIN TO BE ADMINISTERED TO CASES OF VARYING GRADES OF SEVERITY
(After Park.)

Age or Weight of Patient.	Mild Cases.	Early Moderate.	Late Moderate and Early Severe.	Severe and Malignant
Under 2 years. 10-30 lb. wt.	2,000-3,000	3,000-5,000	5,000-10,000	7,500-10,000
2-15 years. 30-90 lb. wt.	3,000-4,000	4,000-10,000	10,000-15,000	10,000-20,000
Adults. 90 lb. or over	3,000-5,000	5,000-10,000	10,000-20,000	20,000-50,000
Route of administration.	Intramuscular	Intravenous	Intravenous	Intravenous

It might be expected that after some 50 years it would be easy to produce irrefutable statistical evidence of the beneficial effects of the antitoxin treatment of diphtheria in man. The evidence does, in truth, seem decisive to most of us; that it is still possible to bring forward contrary arguments that are not altogether specious is due to the fact that once a strong presumptive case has been made out in favour of a particular therapeutic measure it is not justifiable to continue the period of trial at a risk of human lives.

Of the classical studies from the early days of antitoxin treatment, the only one that is still available is that of Park (1921). For a period of 100 days, 239 cases were divided into two groups by separating those admitted on alternate days. All cases admitted on one day were given antitoxin, all those admitted on the next day were treated without antitoxin, and so on throughout the period of trial. The results were as follows: of 239 cases treated with antitoxin 8 died, a fatality of 3.5 per cent.; of 245 cases treated without antitoxin 30 died, a fatality of 12.25 per cent. Applying the formula given on p. 1124, the difference between the case fatality in the two groups is 8.75 per cent. and the standard error of this difference is 2.445 per cent., so that the odds against the difference being due to random sampling are several thousands to one (see Table 59, p. 1122).

Other results recorded during the early and middle 'nineties—comparisons between the case-fatality rates in hospitals in which antitoxin was given and others in which it was not, or between the experience in one hospital before and after the introduction of antitoxin—all pointed in the same direction; and the period of trial ended with the adoption of antitoxin as a routine method of treatment.

If we survey the course of events since that time we find that they strongly support the view that the use of antitoxin has resulted in a significant lowering of

THE PNEUMOCOCCI

The bacterium most commonly found in pneumonia in man is a small lancet-shaped micrococcus which has been variously termed *Micrococcus pneumoniae*, *Micrococcus lanceolatus*, *Streptococcus pneumoniae*, or more briefly, the *pneumococcus* or *Frankel's pneumococcus*. *Diplococcus pneumoniae* is now the commonly accepted formal name.

THE INCIDENCE OF ETIOLOGIC AGENTS IN PNEUMONIA*

Causative Organism	Lobar Pneumonia	Broncho- pneumonia	Unspecified	All Pneu- monias
<i>Pneumococcus</i>	82.48	65.79	77.48	77.71
Hemolytic streptococcus	2.00	3.33	3.99	2.65
Other streptococci	1.30	2.99	1.33	1.70
Staphylococcus	.82	2.00	1.38	1.19
Friedlander's bacillus	.15	.13	.28	.17
Influenza bacillus	.06	.25	.11	.15
Tubercle bacillus		.08		.02
Fungi		.02		
Virus			.07	.01
No significant organism re- corded	13.19	25.41	15.38	16.44
Number of cases	15,420	6,092	4,290	25,802

* In six representative states over a two-year period as compiled by Rumreich *et al.*, Pub. Health Repts., 1943, 58:121.

Of the generally recognized anatomical types of pneumonia, lobar or acute croupous pneumonia, bronchopneumonia or lobular pneumonia, and capillary bronchitis or bronchiolitis, lobar pneumonia is nearly always due to the pneumococcus, though other bacteria are occasionally involved. Perhaps the best quantitative data are those assembled by Rumreich and his co-workers¹ in a two-year study in six states representing high and low pneumonia rates. These are summarized in the accompanying table. It will be clear that the pneumococcus is by far the commonest cause of pneumonia. The microorganisms associated with bronchopneumonia are varied (see table) and their source is probably almost always the nasopharynx.² Pneumonia is a relatively common disease, 101,811 cases and 34,421 deaths were reported in 1945 by 31 states, rates of 124.3 and 42.0 per 100,000 population respectively.

¹ Rumreich *et al.*, Pub. Health Repts., 1943, 58:121.

² See Smilie and Duerschner: Amer. Jour. Hyg., 1947, 45:1, 13.

factors, such as changes in the proportion of all laryngeal cases treated by tracheotomy, and in the period at which tracheotomy is performed, make it difficult to assess the real significance of the recorded figures.

In any case, the clear historical evidence of long-period fluctuations in the severity of diphtheria, as of other infective diseases, forbids us to place too great an emphasis on a progressive fall in mortality lasting for a few decades; and, in recent years, there have been signs that the disease is still able to reassert its killing power. In Berlin, for instance, the case-fatality rate in the Rudolf Virchow Hospital rose in 1926 to 17.4 per cent. (Deicher and Agulnik 1927), and similar experiences have been recorded elsewhere. How far this increase was due to severe infections of the *gravis* type, it is impossible to say, since type-differentiation of diphtheria bacilli is of comparatively recent origin. Another possibility that has to be borne in mind is that the modern processes of serum purification are removing some antitoxic or other factor present in natural serum which helps to control the disease. Clinicians are disturbed at the failure of severe cases of diphtheria to respond to even enormous doses of antiserum, and there is some reason to believe that the refined product of to-day is less potent in combating toxæmia than the crude serum used earlier in the century.

We are thus left with a mass of statistical data all of which is compatible with the view that the use of antitoxin has had a considerable effect in lowering the mortality from diphtheria, but most of which is indecisive. Taking the general trend of this evidence with Fibiger's early observations and adding to it the decisive results obtained in animal experiments, we have a sound basis for the conclusion that antitoxin provides the best available method of treatment, and that its early administration is the essential factor in controlling the clinical disease. The failure to establish a statistical case that is beyond criticism merely shows how hard it is to assess the real effect of any remedy in any human disease, unless that effect is so dramatic as to be immediately and consistently evident.

In view of recent experience, in Germany, in England and elsewhere, there is, however, an obvious need to re-examine the problem with a view to improving, or adding to, our present therapeutic reagents.

The Standardization of Diphtheria Antitoxin

We have already referred to the standardization of diphtheria antitoxin, in our discussion of the toxin-antitoxin reaction (pp 273-7). We noted that the instability of a toxic filtrate renders it quite unsuitable as a standard of reference, and that, for this reason among others, Ehrlich's original definition of the unit of antitoxin as *the smallest amount of antitoxin that will neutralize 100 M.L.D. of toxin*, using the guinea-pig as the test animal, had soon to be abandoned, and has been replaced by a unit defined in terms of a standard antitoxin. Such a standard antitoxic serum, when dried and preserved *in vacuo* in the presence of phosphorus pentoxide, maintains its potency over long periods of time. It has, moreover, become a general principle in biological standardization that a reagent shall, wherever possible, be assayed by comparing its potency with that of a standard preparation of the same reagent, to which some unit value has been assigned by international agreement (see p 1134). In the case of diphtheria, Ehrlich's original antitoxin was adopted as the first international standard preparation, and subsequent standards have been characterized in terms of the first (see Report 1923). so that the unit of activity remains constant, though the actual weight of the

The pneumococcus was discovered independently in France in 1881 by Pasteur, who inoculated rabbits with the saliva of a child dead of rabies, and by Sternberg in the United States, but was not known to be associated with disease in man before the extensive investigations of Frankel and Weichselbaum, who demonstrated conclusively the etiological relation of this bacterium to pneumonia in man.

Morphology and Staining.³ The pneumococcus is typically a small, slightly elongated coccus, one end of which is pointed or lance-shaped. The cocci commonly occur in pairs (diplococci), but variations both in grouping and in size and form of individual cells are frequently observed. Chain formation is common, especially in artificial media, although the chains are usually shorter than those of *Streptococcus pyogenes*. Oval and elongated bacillary forms sometimes occur. The pneumococcus is non-motile and does not form spores. A well-defined capsule envelops the pneumococci in animal

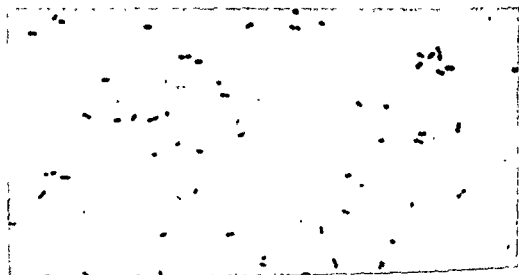


Fig 51. *Pneumococcus*, pure culture. Note the typical lanceolate shape and diplococcus arrangement. Fuchsin; $\times 1050$.

exudates but, except in certain strains or in certain media, is less readily demonstrable in cultures grown outside the body. Capsules may often be found in milk cultures and in media containing blood or serum.

The pneumococcus is readily stained with the aniline dyes and is generally gram positive, although there is a tendency to become gram-negative in older cultures and occasional strains are found to be gram-negative. In stained preparations the capsule may often be seen as an unstained halo surrounding the cells; it may be stained by special methods.

The colonies of pneumococcus on infusion agar or blood agar are typically small, moist, translucent and granular, with well-defined edges. These bacteria are α hemolytic on blood agar, and the colonies appear surrounded by a zone of greenish discoloration on this medium and resemble the colonies of green-producing streptococci.

Physiology. Some pneumococci grow upon the ordinary nutrient (beef extract) culture media but many do not, and in any case growth is sparse. Nu

³ The pneumococcus is completely and exhaustively discussed by White: *The Biology of Pneumococcus*. The Commonwealth Fund, New York. 1938.

mixture that shows optimal flocculation when tested by the Ramon method should be called the Lf dose. The procedure with this *in vitro* test is essentially the same as with either of the *in vivo* methods. The Lf dose of a suitable toxic filtrate is determined by titration against the standard antitoxin. The amount of the serum under test that gives optimal flocculation with the Lf dose of toxin is determined by a second titration. This amount of serum contains one unit of antitoxin.

We may set out the units or named doses employed in testing diphtheria toxin and antitoxin as follows (see Glenny 1925):

- (1) Toxin is measured by its direct toxic effect in the guinea-pig as
M.L.D. (death on the 4th day)
M.R.D. (minimal skin reaction).
There is no fixed equivalent amount of antitoxin.
- (2) Combining power with one unit of antitoxin is measured in the guinea-pig as
L+ dose (mixture causes death on the 4th day)
Lo (mixture causes minimal oedema)
Lr (mixture causes minimal skin reaction).
- (3) Combining power with one unit of antitoxin is measured *in vitro* as
Lf (mixture gives most rapid flocculation in Ramon test).

The comparison of large numbers of different antitoxic sera—natural or concentrated—by these different methods has brought to light facts of considerable theoretical and practical importance. The L+ dose of toxin is always appreciably larger than the Lo dose. The Lr dose of toxin always approximates closely to the Lo dose, as would be expected, since a very small excess of unneutralized toxin will elicit the Romer reaction. The Lf dose is, in general, slightly less than the Lr dose. Glenny (1925) notes the following relation between the various doses of an average toxic filtrate: L+ dose = 0.21 ml., Lo dose = 0.18 ml., Lr dose = 0.175 ml., Lf dose = 0.155 ml. The ratio of the Lf dose to any of the *in vivo* doses is not constant for all toxic filtrates. Antitoxin gives flocculation with both toxin and toxoid, while the determination of the L+, Lo and Lr doses depends on the presence of unneutralized toxin, and is hence affected by differences in the proportion of toxin to toxoid. Moreover, as was shown by Glenny, Pope and Waddington (1925), not only does the Lf/Lr ratio vary from one toxic filtrate to another, when these are tested against the same serum, but the Lf/Lr ratio of a single toxic filtrate varies when it is tested against different antitoxic sera. It follows that, when sera are compared with one another by *in vivo* and *in vitro* methods, their apparent relative potency may vary according to the method of comparison employed; and, with such sera, the ratio $\frac{\text{in vitro value}}{\text{in vivo value}}$ will vary from one serum to another. Glenny and

his colleagues note that they have obtained *in vitro/in vivo* ratios varying from 0.4 to 2.0 with different antitoxic sera, and that, in general, if the Ehrlich value is considerably higher than the Ramon value, i.e., if the *in vitro/in vivo* ratio is low, the serum will be found to give rapid flocculation; whereas, if the Ramon value is higher than the Ehrlich value, the serum will be found to give very slow flocculation and the toxin-antitoxin complex will show considerable dissociation on simple dilution. In their later papers, Glenny and his colleagues use the inverse ratio—*in vivo/in vitro*—so that a serum with a ratio greater than unity has a greater protective action than its flocculation value would lead one to expect.

The inverse ratio is a measure of avidity, a quality of antitoxin which we discussed on p. 277. There we noted that avidity can also be measured by the dilution ratio, which, like the serum ratio, was introduced by Glenny and his colleagues. This ratio is the amount of antitoxin required to neutralize the L+ dose of toxin in a total volume of 2 ml., divided by the amount necessary to neutralize this dose in a volume of 200 ml., as determined by the guinea-pig intracutaneous test. These levels of testing correspond to the Lr/10 and

tritional requirements are complex; Rane and Subbarow⁴ have been able to grow Types 1, 2, 5 and 8 but not Type 7 in a medium consisting of acid hydrolysate of gelatin supplemented with glutamic acid, cystine, glucose, pantothenic acid, nicotinic acid, choline and thioglycollic acid. Similar observations have been reported by other workers.⁵ Growth on infusion media, particularly those enriched by the addition of whole blood, takes place at 37° C. Litmus milk is promptly acidified and often, but not invariably, coagulated. The temperature range over which these bacteria may be grown is relatively narrow (25° to 42° C.), and they are sensitive to variations in pH from the optimum of 7.8, the limiting acidity and alkalinity being 7.0 and 8.3 respectively. The pneumococcus is a facultative anaerobe, although certain other species of *Diplococcus* are obligate anaerobes.

In general, sugars are actively fermented with the production of large amounts of lactic acid and small amounts of volatile acid and ethyl alcohol.

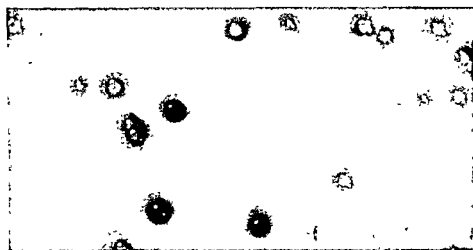


Fig. 52. Colonies of the pneumococcus on blood agar. The areas of green hemolysis have been accentuated in the photograph $\times 3$.

Differential fermentations are of no particular value in the classification of these microorganisms except in the case of inulin, which serves to differentiate the

mt. autolyze
of protein
hydrolysis accompany autolysis, and it appears that lysis is a consequence of the activity of intracellular ferments. Perhaps associated with the autolytic process is the lysis of pneumococci by bile and bile salts. The so-called "bile solubility" of pneumococci is practically a constant characteristic, although different strains vary in their sensitivity to bile as they do in the tendency to autolysis. Rabbit or ox bile may be used and added to a young broth culture in the proportion of 10 to 20 per cent. Solutions of pure bile salt (sodium taurocholate) are preferable to ox bile because they may be sterilized and the concentration

⁴ Rane and Subbarow: Jour. Bact., 1940, 40:695.

⁵ Gilbert. Proc. Soc. Exp. Biol. Med., 1944, 57:363, Adams and Roe. Jour. Bact., 1945, 49:401.

prophylaxis or therapy. It happens that high-potency antitoxins are usually avid, and can be relied on in this respect. There are, however, no agreed measures of avidity by which therapeutic antitoxins can be specified (see, e.g., British Pharmacopoeia 1953). Jerne (1951) suggests the determination of a single association constant for each antitoxin, to be designated an "avidity constant," and describes a method for its determination, but the practicability of this proposal remains to be established.

The Standardization of Diphtheria Prophylactics

The flocculation test of Ramon (1922) provides an obvious method of determining the amount of toxoid in any prophylactic, and Glenny, Pope and Waddington (1925) found that the immunizing value of a modified toxin was closely related to its Lf value. It has, indeed, become a common practice to state the dose of toxoid contained in a prophylactic in terms of Lf units, when the actual dose of toxoid is stated at all, which is not often the case. But it would obviously be preferable, were it possible, to measure the actual immunizing potency of all preparations by direct comparison with some arbitrarily selected standard reagent.

The technical differences involved in such a comparison are, however, very great. For the reasons discussed in Chapter 43, a comparison carried out on a small number of animals is quite valueless; and it is doubtful whether the precise standardization of a reagent used for inducing active immunity is as yet within the realm of practical politics.

The relevant Regulations (1952) in force in Great Britain, under the Therapeutic Substances Act, may be summarized as follows.

Toxicity. Five ml. of formol toxoid, T.A.M. or T.A.F., and 1 ml. of A.P.T. or P.T.A.P., are injected subcutaneously or intraperitoneally into each of not less than 5 healthy guinea-pigs, each weighing from 250 to 350 gm. The injection must not cause the death of any of the guinea-pigs from specific intoxication within 20 days.

Immunizing Potency. The test for minimum immunizing potency of formol toxoid consists in the injection into each of 10 healthy guinea-pigs of 0.05 ml. of F.T. in 1 ml. saline, on 2 occasions at an interval of not more than 4 weeks. Not later than 3 weeks after the second injection each guinea-pig receives an intracutaneous injection of 0.2 ml. of a dilution of diphtheria toxin containing 5 Schick-test doses. If, 48 hours later, more than 2 of the 10 guinea-pigs exhibit a positive Schick reaction, the preparation is insufficiently potent. T.A.M. and T.A.F. are tested similarly, except that T.A.M. inoculated animals are tested with 1 Schick-test dose; and in the T.A.F. test, 9 animals are used, and tested with 1 and 2 Schick-test doses. In the last case, no more than 1/3rd of the animals must react to 1 Schick-test dose, or alternatively, no more than 2/3rd to 2 Schick test doses. For the purposes of the tests, a Schick-test dose contains 0.001 Lf and free toxin in an amount such that one twentieth (0.0005 Lf) or less given intracutaneously into guinea-pigs will, after 48 hours, regularly produce a local reaction of the Schick positive type.

A.P.T. and P.T.A.P. are diluted in saline so that the equivalent of 1 Lf is present in 1 ml. Each of 10 guinea-pigs receives 1 ml. on two occasions at an interval of not more than 4 weeks. Not later than 3 weeks after the second injection, the antitoxin content of the serum of each animal is determined. If the geometric mean of the antitoxin contents is less than 2 units, the preparation is insufficiently potent (see also British Pharmacopoeia 1953, p. 200).

These tests, it will be noted, prescribe limits of toxicity and antigenic efficiency, and standardization in terms of units. Their reliability depends in part upon the maintenance of guinea-pigs of an average susceptibility to toxin and to immunizing agents. Since we cannot know how far laboratory guinea-pigs vary in these respects, the requirements are devised so that the antigenic content of prophylactics that pass the tests is in substantial excess of that necessary for good immunization. The alternative, as we saw in Chapter 43, is to express potency in terms of standards. Although for many years Prügge (1939) in Germany used a standard preparation of diphtheria prophylactic, and

(10 per cent) controlled. Heat-killed pneumococci are not bile-soluble. Sodium lauryl sulfate and similar detergents will also lyse pneumococci.

Pneumococci may, therefore, be distinguished from streptococci by their bile-solubility and, less definitely, their ability to ferment inulin, in addition to their greater pathogenicity for mice and the characteristics of the colonies on agar.

Peroxide is found in considerable quantities of pneumococcus cultures after prolonged incubation because of the lack of catalase in these microorganisms. This, coupled with their sensitivity to peroxide, results in the auto-sterilization of cultures kept in the incubator for many days. Cultures in blood broth, however, remain viable for several weeks in the refrigerator and the bacteria may be preserved for months in the cold in vacuum-desiccated spleens of infected mice.

The pneumococci are more sensitive to the bactericidal activity of the usual antiseptics than are many other bacteria. Soaps, such as ricinoleate and oleate, are pneumococcicidal in relatively high dilutions, 0.04 and 0.004 per cent respectively, and other substances such as phenol and mercuric chloride are also highly effective in the destruction of these bacteria. Quinine and some of its derivatives, such as optochin, are also pneumococcicidal, a fact which has been of interest in connection with the chemotherapy of pneumococcus pneumonia.

Toxins. The severe intoxication observed in pneumococcus infection in man is suggestive of the formation of some toxin by this bacterium. The existence of such a toxin has never been demonstrated, however, and the pneumococcus does not produce a toxin analogous to those of the diphtheria and tetanus bacilli.

Other toxic substances are produced by this microorganism. That there is a hemolysis on blood plates has already been noted, and there is in addition a filterable hemolysin active on sheep, guinea pig and human erythrocytes. The concentrated hemolysin is reported to have lethal and dermatotoxic properties.⁶ The pneumococcus also produces a leucocidin and a necrotizing substance similar to that formed by some of the staphylococci. Many strains produce hyaluronidase, especially when cultivated in media containing hyaluronic acid.⁷ A purpura-producing substance which is non-antigenic and appears to be a cleavage product of pneumococcal protein has been described by a number of workers. Injected into white mice, extracts of pneumococci produce a purpuric condition manifested as a dark blue discoloration of the skin of the feet, tail, ears, nose and genitals.⁸

Although preparations containing these activities have been reported to increase the virulence of relatively avirulent pneumococcus strains when injected simultaneously with the bacteria, the virulence of pneumococci is directly dependent, not on the formation of such toxic substances, but on the production of specific soluble substance and encapsulation.

Classification. The pneumococci are closely related to the streptococci, but the degree of intimacy of the relationship is as yet open to question. Some workers regard these microorganisms as but a species of streptococcus and design

⁶ Halbert, Cohen and Perkins. *Bull. Johns Hopkins Hosp.*, 1946, 78 340.

⁷ See Humphrey. *Jour. Path. Bact.*, 1944, 56 273.

⁸ Cf. Julianelle and Reimann. *Jour. Exp. Med.*, 1926, 43 87, *ibid.*, 1927, 45 609.

sites of pathological lesions in man. They have been cultivated from the blood of typhus cases, from the cerebrospinal fluid of cases of general paralysis, from the conjunctiva in various forms of subacute or chronic conjunctivitis, from the external auditory meatus in cases of ear disease, from the pustules of acne vulgaris, from other lesions of the skin, from the urine in cases of subacute or chronic urethritis, and from lymphatic glands in a variety of diseases, particularly lymphadenoma. In a few instances, including perhaps acne vulgaris, the evidence suggests that the association is a causative one, but in most cases the early claims to have established an aetiological relationship have broken down in the light of subsequent investigations. There is, as yet, no satisfactory evidence that any corynebacterium, other than *C. diphtheriae*, with the possible exception of *C. acnes*, plays any significant rôle as a pathogenic parasite of man; though various species form an important constituent of his normal bacterial flora (Andrewes *et al.* 1923, Harris and Wade 1915).

Diphtheroid Infections in Animals.

There are a few diseases which occur naturally in animals other than man, and are caused by infection with corynebacteria.

Ulcerative Lymphangitis of Horses and Pseudotuberculosis of Sheep.—Both these diseases are the result of infection with *C. oris*, or, as it is sometimes called, the Preisz-Nocard bacillus. The characters of this organism and the lesions that it produces in experimental animals have been described in Chapter 17. It may be noted that this organism, unlike *C. diphtheriae*, is pyogenic and invasive, as well as toxigenic. The exotoxin produced by it differs from the exotoxin of *C. diphtheriae* both in the character of the lesions produced in experimental animals and in its antigenic relationships. The pathogenesis of the natural disease in horses and sheep seems to be determined mainly by the invasive and pyogenic activities of the causative organism. The part played by the toxin is at present doubtful. Pseudotuberculosis of sheep—better known perhaps as *caseous lymphadenitis*—is economically an important disease, particularly in Australia (see Bull and Dickinson 1931, 1933, 1935, Dickinson and Bull 1931, Discussion 1934). It is a chronic disease characterized by caseous abscesses. The lymph glands are mainly affected, but the lungs and sometimes the testicles may suffer too. The wool becomes dry and lifeless. The abscesses range in size from a millimetre or so in diameter up to 3 or 4 inches; they are rather gritty and contain greenish pus. Infection occurs through wounds of the skin such as occur in shearing, docking and castration.

Infections caused by *C. pyogenes*.—This organism is probably the most important and widespread member of the *Corynebacterium* group found in association with animal disease. It is responsible for suppurative processes, including pneumonia, arthritis, and mastitis in cattle, pigs, sheep, and goats. A weak toxin is formed, which often gives rise to antitoxin production in infected animals; this may be measured by the power of the serum to inhibit the hæmolysis of rabbit cells by the toxin (Lovell 1939). The toxin may be converted into toxoid by treatment with formalin. An alum-precipitated toxoid induces antitoxin formation in animals inoculated intramuscularly, but early field trials did not prove very hopeful (Lovell *et al.* 1950).

Pyæmia in Foals.—Magnusson (1923, 1933) described a pyæmic disease of foals in Sweden due to a pigment-forming organism, which he called *C. equi* (see p. 547). As a rule the disease is characterized by a suppurative bronchopneumonia

nate them as *Streptococcus pneumoniae*. In general, however, it is customary to consider the pneumococci as a distinct genus, a practice which is justified by considerations of the sum of the characteristics of the pneumococci and the clinical and epidemiological aspects of the pneumonias. According to Bergey's (1948) classification, the tribe *Streptococcaceae* is made up of three genera (1) *Diplococcus*, of which the type species is *Diplococcus pneumoniae* or the pneumococcus, (2) *Streptococcus*, with *Streptococcus pyogenes* as the type species, and (3) *Leuconostoc*, a group of gas-forming, chain-producing cocci found in milk, fermenting vegetables and slimy sugar solutions.

The genus *Diplococcus* includes six species in all, the five in addition to the pneumococcus being obligate anaerobes. *D. paleopneumoniae* closely resembles

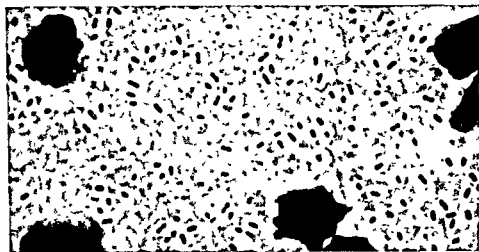


Fig. 53. Pneumococcus in the peritoneal fluid of a mouse. Note the capsules. Fuchsin, $\times 2200$.

the pneumococcus except for its anaerobic character, occurs normally in the buccal-pharyngeal cavity, and is reported to be highly pathogenic. *D. plagarum-belli* has been found in septic wounds, and the remainder, *D. magnus*, *D. constellatus* and *D. morbillorum*, appear to be normal inhabitants of the mouth and intestinal tract, and have been found in lymphoid tissue such as in the tonsils and appendix.

Pneumococcus Types. A point that has been of somewhat more interest than the formal taxonomic position of the pneumococci is the subdivision of these bacteria into types. The pneumococci contain two types of antigens. One, the so-called somatic antigen, is a constituent of the cell substance proper and is immunologically identical in all pneumococci. The other, the polysaccharide haptene or specific soluble substance (SSS), is type specific and serves to differentiate the immunological types of pneumococci from one another. The polysaccharide of Type 3 has been the most thoroughly studied chemically (p. 282) and Types 1 and 2 to a lesser extent. These substances have been isolated from most of the other types also. The presence of SSS masks the somatic antigen, and antisera to encapsulated pneumococci are sharply type-specific. These immunological types are sometimes designated by Roman nu-

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merals, and the numbering of the types, although purely arbitrary, is a universally accepted convention.

Observations by Dochez and Gillespie⁹ showed that the pneumococci found in cases of lobar pneumonia can be divided into four distinct groups, designated as Types 1, 2 and 3 and Group IV, on the basis of specific agglutination and protection tests. Of these, Types 1, 2 and 3, found in the majority of cases of pneumonia (Fig. 54) and producing the most severe types of infection, have specific immunological character, while Group IV is immunologically heterogeneous and is made up of all pneumococci not belonging to the first three types. Through the work of Cooper and her associates of the New York City Department of Health¹⁰ 29 additional serologic types were sorted out of Group IV to make 32 types in all. Of these 26 is closely related to 6 and is sometimes designated 6a, and 15 and 30 are identical or practically so. Six new types and 14 subtypes, related to but not identical with the Cooper types, were described by Kauffmann, Mørch and Schmith¹¹ who pointed out that the subtypes, while related, were stable and independent. This differentiation was made on the basis of reciprocal absorptions. Seventeen additional types were described by Walter, Guevin, Beattie, Colter and Bucca¹² of which 12 corresponded to 5 of the types and 7 of the subtypes of Kauffmann *et al.* Mørch¹³ then later described additional new types, some of which were subtypes. The practice of designating subtypes, viz., 7a, 7b, 7c and so on, has begun to be generally adopted. Under the auspices of the American Public Health Association and the United States Public Health Service, however, Eddy¹⁴ has given individual type numbers to all pneumococcus types and subtypes so far described without regard to the relationships, sometimes very close, between many of the types, making a total of 75 types. Thus the purely arbitrary system of numbering the types without regard to their biological relationships is reverted to and extended.

Although these immunological types are readily distinguished from one another, cross-reactions occur in a number of instances. There appear to be some relations between Types 2 and 5, 3 and 8, 7 and 18, and 15 and 30 which are quite likely due to structural similarities of the polysaccharide haptens (p. 282). The resemblances are not sufficiently close, however, to invalidate the differentiation of these types. An interesting observation has been that of Forster and Shaughnessy¹⁵ of the occurrence of mixed types, i.e., strains of pneumococci which react with two or more unrelated type specific antisera. Similar observations have been reported by Chinn and Eddy.¹⁶ Such mixed types appear to occur with some frequency as indicated in Fig. 54.

Diplococcus Mucosus. These immunological types are culturally indis-

⁹ Dochez and Gillespie: Jour. Amer. Med. Assn., 1913, 61:727.

¹⁰ Cooper, Edwards and Rosenstein: Jour. Exp. Med., 1929, 49:461; Cooper, Rosenstein, Walter and Peizer: Jour. Exp. Med., 1932, 55:531.

¹¹ Kauffman, Mørch and Schmith: Jour. Immunol., 1940, 39:397.

¹² Walter, Guevin, Beattie, Colter and Bucca: Jour. Immunol., 1941, 41:279.

¹³ Mørch: Jour. Immunol., 1942, 43:177.

¹⁴ Eddy: Pub. Health Repts., 1944, 59:449, 451, 485.

¹⁵ Forster and Shaughnessy: Proc. Soc. Exp. Biol. Med., 1940, 44:306.

¹⁶ Chinn and Eddy: Pub. Health Repts., 1941, 56:62.

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(1955l) *Ibid.*, No. 123; (1955m) *Ibid.*, No. 124; (1955n) *Ibid.*, No. 125; (1955o) *Ibid.*, No. 126; (1955p) *Ibid.*, No. 127; (1955q) *Ibid.*, No. 128; (1955r) *Ibid.*, No. 129; (1955s) *Ibid.*, No. 130; (1955t) *Ibid.*, No. 131; (1955u) *Ibid.*, No. 132; (1955v) *Ibid.*, No. 133; (1955w) *Ibid.*, No. 134; (1955x) *Ibid.*, No. 135; (1955y) *Ibid.*, No. 136; (1955z) *Ibid.*, No. 137; (1956a) *Ibid.*, No. 138; (1956b) *Ibid.*, No. 139; (1956c) *Ibid.*, No. 140; (1956d) *Ibid.*, No. 141; (1956e) *Ibid.*, No. 142; (1956f) *Ibid.*, No. 143; (1956g) *Ibid.*, No. 144; (1956h) *Ibid.*, No. 145; (1956i) *Ibid.*, No. 146; (1956j) *Ibid.*, No. 147; (1956k) *Ibid.*, No. 148; (1956l) *Ibid.*, No. 149; (1956m) *Ibid.*, No. 150; (1956n) *Ibid.*, No. 151; (1956o) *Ibid.*, No. 152; (1956p) *Ibid.*, No. 153; (1956q) *Ibid.*, No. 154; (1956r) *Ibid.*, No. 155; (1956s) *Ibid.*, No. 156; (1956t) *Ibid.*, No. 157; (1956u) *Ibid.*, No. 158; (1956v) *Ibid.*, No. 159; (1956w) *Ibid.*, No. 160; (1956x) *Ibid.*, No. 161; (1956y) *Ibid.*, No. 162; (1956z) *Ibid.*, No. 163; (1957a) *Ibid.*, No. 164; (1957b) *Ibid.*, No. 165; (1957c) *Ibid.*, No. 166; (1957d) *Ibid.*, No. 167; (1957e) *Ibid.*, No. 168; (1957f) *Ibid.*, No. 169; (1957g) *Ibid.*, No. 170; (1957h) *Ibid.*, No. 171; (1957i) *Ibid.*, No. 172; (1957j) *Ibid.*, No. 173; (1957k) *Ibid.*, No. 174; (1957l) *Ibid.*, No. 175; (1957m) *Ibid.*, No. 176; (1957n) *Ibid.*, No. 177; (1957o) *Ibid.*, No. 178; (1957p) *Ibid.*, No. 179; (1957q) *Ibid.*, No. 180; (1957r) *Ibid.*, No. 181; (1957s) *Ibid.*, No. 182; (1957t) *Ibid.*, No. 183; (1957u) *Ibid.*, No. 184; (1957v) *Ibid.*, No. 185; (1957w) *Ibid.*, No. 186; (1957x) *Ibid.*, No. 187; (1957y) *Ibid.*, No. 188; (1957z) *Ibid.*, No. 189; (1958a) *Ibid.*, No. 190; (1958b) *Ibid.*, No. 191; (1958c) *Ibid.*, No. 192; (1958d) *Ibid.*, No. 193; (1958e) *Ibid.*, No. 194; (1958f) *Ibid.*, No. 195; (1958g) *Ibid.*, No. 196; (1958h) *Ibid.*, No. 197; (1958i) *Ibid.*, No. 198; (1958j) *Ibid.*, No. 199; (1958k) *Ibid.*, No. 200; (1958l) *Ibid.*, No. 201; (1958m) *Ibid.*, No. 202; (1958n) *Ibid.*, No. 203; (1958o) *Ibid.*, No. 204; (1958p) *Ibid.*, No. 205; (1958q) *Ibid.*, No. 206; (1958r) *Ibid.*, No. 207; (1958s) *Ibid.*, No. 208; (1958t) *Ibid.*, No. 209; (1958u) *Ibid.*, No. 210; (1958v) *Ibid.*, No. 211; (1958w) *Ibid.*, No. 212; (1958x) *Ibid.*, No. 213; (1958y) *Ibid.*, No. 214; (1958z) *Ibid.*, No. 215; (1959a) *Ibid.*, No. 216; (1959b) *Ibid.*, No. 217; (1959c) *Ibid.*, No. 218; (1959d) *Ibid.*, No. 219; (1959e) *Ibid.*, No. 220; (1959f) *Ibid.*, No. 221; (1959g) *Ibid.*, No. 222; (1959h) *Ibid.*, No. 223; (1959i) *Ibid.*, No. 224; (1959j) *Ibid.*, No. 225; (1959k) *Ibid.*, No. 226; (1959l) *Ibid.*, No. 227; (1959m) *Ibid.*, No. 228; (1959n) *Ibid.*, No. 229; (1959o) *Ibid.*, No. 230; (1959p) *Ibid.*, No. 231; (1959q) *Ibid.*, No. 232; (1959r) *Ibid.*, No. 233; (1959s) *Ibid.*, No. 234; (1959t) *Ibid.*, No. 235; (1959u) *Ibid.*, No. 236; (1959v) *Ibid.*, No. 237; (1959w) *Ibid.*, No. 238; (1959x) *Ibid.*, No. 239; (1959y) *Ibid.*, No. 240; (1959z) *Ibid.*, No. 241; (1960a) *Ibid.*, No. 242; (1960b) *Ibid.*, No. 243; (1960c) *Ibid.*, No. 244; (1960d) *Ibid.*, No. 245; (1960e) *Ibid.*, No. 246; (1960f) *Ibid.*, No. 247; (1960g) *Ibid.*, No. 248; (1960h) *Ibid.*, No. 249; (1960i) *Ibid.*, No. 250; (1960j) *Ibid.*, No. 251; (1960k) *Ibid.*, No. 252; (1960l) *Ibid.*, No. 253; (1960m) *Ibid.*, No. 254; (1960n) *Ibid.*, No. 255; (1960o) *Ibid.*, No. 256; (1960p) *Ibid.*, No. 257; (1960q) *Ibid.*, No. 258; (1960r) *Ibid.*, No. 259; (1960s) *Ibid.*, No. 260; (1960t) *Ibid.*, No. 261; (1960u) *Ibid.*, No. 262; (1960v) *Ibid.*, No. 263; (1960w) *Ibid.*, No. 264; (1960x) *Ibid.*, No. 265; (1960y) *Ibid.*, No. 266; (1960z) *Ibid.*, No. 267; (1961a) *Ibid.*, No. 268; (1961b) *Ibid.*, No. 269; (1961c) *Ibid.*, No. 270; (1961d) *Ibid.*, No. 271; (1961e) *Ibid.*, No. 272; (1961f) *Ibid.*, No. 273; (1961g) *Ibid.*, No. 274; (1961h) *Ibid.*, No. 275; (1961i) *Ibid.*, No. 276; (1961j) *Ibid.*, No. 277; (1961k) *Ibid.*, No. 278; (1961l) *Ibid.*, No. 279; (1961m) *Ibid.*, No. 280; (1961n) *Ibid.*, No. 281; (1961o) *Ibid.*, No. 282; (1961p) *Ibid.*, No. 283; (1961q) *Ibid.*, No. 284; (1961r) *Ibid.*, No. 285; (1961s) *Ibid.*, No. 286; (1961t) *Ibid.*, No. 287; (1961u) *Ibid.*, No. 288; (1961v) *Ibid.*, No. 289; (1961w) *Ibid.*, No. 290; (1961x) *Ibid.*, No. 291; (1961y) *Ibid.*, No. 292; (1961z) *Ibid.*, No. 293; (1962a) *Ibid.*, No. 294; (1962b) *Ibid.*, No. 295; (1962c) *Ibid.*, No. 296; (1962d) *Ibid.*, No. 297; (1962e) *Ibid.*, No. 298; (1962f) *Ibid.*, No. 299; (1962g) *Ibid.*, No. 300; (1962h) *Ibid.*, No. 301; (1962i) *Ibid.*, No. 302; (1962j) *Ibid.*, No. 303; (1962k) *Ibid.*, No. 304; (1962l) *Ibid.*, No. 305; (1962m) *Ibid.*, No. 306; (1962n) *Ibid.*, No. 307; (1962o) *Ibid.*, No. 308; (1962p) *Ibid.*, No. 309; (1962q) *Ibid.*, No. 310; (1962r) *Ibid.*, No. 311; (1962s) *Ibid.*, No. 312; (1962t) *Ibid.*, No. 313; (1962u) *Ibid.*, No. 314; (1962v) *Ibid.*, No. 315; (1962w) *Ibid.*, No. 316; (1962x) *Ibid.*, No. 317; (1962y) *Ibid.*, No. 318; (1962z) *Ibid.*, No. 319; (1963a) *Ibid.*, No. 320; (1963b) *Ibid.*, No. 321; (1963c) *Ibid.*, No. 322; (1963d) *Ibid.*, No. 323; (1963e) *Ibid.*, No. 324; (1963f) *Ibid.*, No. 325; (1963g) *Ibid.*, No. 326; (1963h) *Ibid.*, No. 327; (1963i) *Ibid.*, No. 328; (1963j) *Ibid.*, No. 329; (1963k) *Ibid.*, No. 330; (1963l) *Ibid.*, No. 331; (1963m) *Ibid.*, No. 332; (1963n) *Ibid.*, No. 333; (1963o) *Ibid.*, No. 334; (1963p) *Ibid.*, No. 335; (1963q) *Ibid.*, No. 336; (1963r) *Ibid.*, No. 337; (1963s) *Ibid.*, No. 338; (1963t) *Ibid.*, No. 339; (1963u) *Ibid.*, No. 340; (1963v) *Ibid.*, No. 341; (1963w) *Ibid.*, No. 342; (1963x) *Ibid.*, No. 343; (1963y) *Ibid.*, No. 344; (1963z) *Ibid.*, No. 345; (1964a) *Ibid.*, No. 346; (1964b) *Ibid.*, No. 347; (1964c) *Ibid.*, No. 348; (1964d) *Ibid.*, No. 349; (1964e) *Ibid.*, No. 350; (1964f) *Ibid.*, No. 351; (1964g) *Ibid.*, No. 352; (1964h) *Ibid.*, No. 353; (1964i) *Ibid.*, No. 354; (1964j) *Ibid.*, No. 355; (1964k) *Ibid.*, No. 356; (1964l) *Ibid.*, No. 357; (1964m) *Ibid.*, No. 358; (1964n) *Ibid.*, No. 359; (1964o) *Ibid.*, No. 360; (1964p) *Ibid.*, No. 361; (1964q) *Ibid.*, No. 362; (1964r) *Ibid.*, No. 363; (1964s) *Ibid.*, No. 364; (1964t) *Ibid.*, No. 365; (1964u) *Ibid.*, No. 366; (1964v) *Ibid.*, No. 367; (1964w) *Ibid.*, No. 368; (1964x) *Ibid.*, No. 369; (1964y) *Ibid.*, No. 370; (1964z) *Ibid.*, No. 371; (1965a) *Ibid.*, No. 372; (1965b) *Ibid.*, No. 373; (1965c) *Ibid.*, No. 374; (1965d) *Ibid.*, No. 375; (1965e) *Ibid.*, No. 376; (1965f) *Ibid.*, No. 377; (1965g) *Ibid.*, No. 378; (1965h) *Ibid.*, No. 379; (1965i) *Ibid.*, No. 380; (1965j) *Ibid.*, No. 381; (1965k) *Ibid.*, No. 382; (1965l) *Ibid.*, No. 383; (1965m) *Ibid.*, No. 384; (1965n) *Ibid.*, No. 385; (1965o) *Ibid.*, No. 386; (1965p) *Ibid.*, No. 387; (1965q) *Ibid.*, No. 388; (1965r) *Ibid.*, No. 389; (1965s) *Ibid.*, No. 390; (1965t) *Ibid.*, No. 391; (1965u) *Ibid.*, No. 392; (1965v) *Ibid.*, No. 393; (1965w) *Ibid.*, No. 394; (1965x) *Ibid.*, No. 395; (1965y) *Ibid.*, No. 396; (1965z) *Ibid.*, No. 397; (1966a) *Ibid.*, No. 398; (1966b) *Ibid.*, No. 399; (1966c) *Ibid.*, No. 400; (1966d) *Ibid.*, No. 401; (1966e) *Ibid.*, No. 402; (1966f) *Ibid.*, No. 403; (1966g) *Ibid.*, No. 404; (1966h) *Ibid.*, No. 405; (1966i) *Ibid.*, No. 406; (1966j) *Ibid.*, No. 407; (1966k) *Ibid.*, No. 408; (1966l) *Ibid.*, No. 409; (1966m) *Ibid.*, No. 410; (1966n) *Ibid.*, No. 411; (1966o) *Ibid.*, No. 412; (1966p) *Ibid.*, No. 413; (1966q) *Ibid.*, No. 414; (1966r) *Ibid.*, No. 415; (1966s) *Ibid.*, No. 416; (1966t) *Ibid.*, No. 417; (1966u) *Ibid.*, No. 418; (1966v) *Ibid.*, No. 419; (1966w) *Ibid.*, No. 420; (1966x) *Ibid.*, No. 421; (1966y) *Ibid.*, No. 422; (1966z) *Ibid.*, No. 423; (1967a) *Ibid.*, No. 424; (1967b) *Ibid.*, No. 425; (1967c) *Ibid.*, No. 426; (1967d) *Ibid.*, No. 427; (1967e) *Ibid.*, No. 428; (1967f) *Ibid.*, No. 429; (1967g) *Ibid.*, No. 430; (1967h) *Ibid.*, No. 431; (1967i) *Ibid.*, No. 432; (1967j) *Ibid.*, No. 433; (1967k) *Ibid.*, No. 434; (1967l) *Ibid.*, No. 435; (1967m) *Ibid.*, No. 436; (1967n) *Ibid.*, No. 437; (1967o) *Ibid.*, No. 438; (1967p) *Ibid.*, No. 439; (1967q) *Ibid.*, No. 440; (1967r) *Ibid.*, No. 441; (1967s) *Ibid.*, No. 442; (1967t) *Ibid.*, No. 443; (1967u) *Ibid.*, No. 444; (1967v) *Ibid.*, No. 445; (1967w) *Ibid.*, No. 446; (1967x) *Ibid.*, No. 447; (1967y) *Ibid.*, No. 448; (1967z) *Ibid.*, No. 449; (1968a) *Ibid.*, No. 450; (1968b) *Ibid.*, No. 451; (1968c) *Ibid.*, No. 452; (1968d) *Ibid.*, No. 453; (1968e) *Ibid.*, No. 454; (1968f) *Ibid.*, No. 455; (1968g) *Ibid.*, No. 456; (1968h) *Ibid.*, No. 457; (1968i) *Ibid.*, No. 458; (1968j) *Ibid.*, No. 459; (1968k) *Ibid.*, No. 460; (1968l) *Ibid.*, No. 461; (1968m) *Ibid.*, No. 462; (1968n) *Ibid.*, No. 463; (1968o) *Ibid.*, No. 464; (1968p) *Ibid.*, No. 465; (1968q) *Ibid.*, No. 466; (1968r) *Ibid.*, No. 467; (1968s) *Ibid.*, No. 468; (1968t) *Ibid.*, No. 469; (1968u) *Ibid.*, No. 470; (1968v) *Ibid.*, No. 471; (1968w) *Ibid.*, No. 472; (1968x) *Ibid.*, No. 473; (1968y) *Ibid.*, No. 474; (1968z) *Ibid.*, No. 475; (1969a) *Ibid.*, No. 476; (1969b) *Ibid.*, No. 477; (1969c) *Ibid.*, No. 478; (1969d) *Ibid.*, No. 479; (1969e) *Ibid.*, No. 480; (1969f) *Ibid.*, No. 481; (1969g) *Ibid.*, No. 482; (1969h) *Ibid.*, No. 483; (1969i) *Ibid.*, No. 484; (1969j) *Ibid.*, No. 485; (1969k) *Ibid.*, No. 486; (1969l) *Ibid.*, No. 487; (1969m) *Ibid.*, No. 488; (1969n) *Ibid.*, No. 489; (1969o) *Ibid.*, No. 490; (1969p) *Ibid.*, No. 491; (1969q) *Ibid.*, No. 492; (1969r) *Ibid.*, No. 493; (1969s) *Ibid.*, No. 494; (1969t) *Ibid.*, No. 495; (1969u) *Ibid.*, No. 496; (1969v) *Ibid.*, No. 497; (1969w) *Ibid.*, No. 498; (1969x) *Ibid.*, No. 499; (1969y) *Ibid.*, No. 500; (1969z) *Ibid.*, No. 501; (1970a) *Ibid.*, No. 502; (1970b) *Ibid.*, No. 503; (1970c) *Ibid.*, No. 504; (1970d) *Ibid.*, No. 505; (1970e) *Ibid.*, No. 506; (1970f) *Ibid.*, No. 507; (1970g) *Ibid.*, No. 508; (1970h) *Ibid.*, No. 509; (1970i) *Ibid.*, No. 510; (1970j) *Ibid.*, No. 511; (1970k) *Ibid.*, No. 512; (1970l) *Ibid.*, No. 513; (1970m) *Ibid.*, No. 514; (1970n) *Ibid.*, No. 515; (1970o) *Ibid.*, No. 516; (1970p) *Ibid.*, No. 517; (1970q) *Ibid.*, No. 518; (1970r) *Ibid.*, No. 519; (1970s) *Ibid.*, No. 520; (1970t) *Ibid.*, No. 521; (1970u) *Ibid.*, No. 522; (1970v) *Ibid.*, No. 523; (1970w) *Ibid.*, No. 524; (1970x) *Ibid.*, No. 525; (1970y) *Ibid.*, No. 526; (1970z) *Ibid.*, No. 527; (1971a) *Ibid.*, No. 528; (1971b) *Ibid.*, No. 529; (1971c) *Ibid.*, No. 530; (1971d) *Ibid.*, No. 531; (1971e) *Ibid.*, No. 532; (1971f) *Ibid.*, No. 533; (1971g) *Ibid.*, No. 534; (1971h) *Ibid.*, No. 535; (1971i) *Ibid.*, No. 536; (1971j) *Ibid.*, No. 537; (1971k) *Ibid.*, No. 538; (1971l) *Ibid.*, No. 539; (1971m) *Ibid.*, No. 540; (1971n) *Ibid.*, No. 541; (1971o) *Ibid.*, No. 542; (1971p)

tinguishable with the exception of Type 3, which stands somewhat apart from the other pneumococci in that it produces a heavy mucoid growth due to its luxuriant capsule formation. It is not infrequently classified as a separate species, *Diplococcus mucosus*. Many cultures show a marked tendency to form chains, and the dividing line between *Diplococcus mucosus* and "*Streptococcus mucosus*" is not a sharp one, if indeed any distinction should be made. These coccoid, heavily capsulated bacteria for the most part ferment inulin and are soluble in bile, so that the tendency is to group them with the pneumococci rather than the streptococci. A few mucoid strains have been reported which are bile insoluble and non-inulin-fermenting.

*Pneumococcus Typing.*¹⁷ The determination of pneumococcus types is a matter of great practical importance to the therapeutic use of antiserum. The various methods that have been developed are basically immunological but differ in technical detail. The immunological procedures are of three kinds: (1) the agglutination of the pneumococci with type-specific antiserum; (2) the precipitation of SSS with type-specific antiserum; and (3) the *Quellung* reaction. The first two have been discussed in previous sections and need not be considered further here. The *Quellung* phenomenon was described by Neufeld in 1902 and since then has come into general use. A suspension of pneumococci is mixed with undiluted antiserum (rabbit serum is preferable to horse serum) on a slide or cover glass, a small amount of Löffler's alkaline methylene blue is added to facilitate observation, and the preparation is examined under the microscope. In the presence of homologous immune serum there is a marked apparent swelling of the capsule without any obvious change in the size of the bacterial cell itself; no such swelling is observed with heterologous sera. The reaction takes place rapidly and the swelling is usually apparent within a few minutes. The nature of the process of swelling is not known; it is of some interest that it may be reversed by the addition of homologous SSS.¹⁸ The use of serum pools considerably facilitates typing, especially the identification of the higher types. The incidence of the various types determines the most advantageous combinations of antisera. A group of combinations often recommended for use in this country is: (a) 1, 2, 7; (b) 3, 4, 5, 6, 8; (c) 9, 12, 14, 15, 17; (d) 10, 11, 13, 20, 22, 24; (e) 16, 18, 19, 21, 28; (f) 23, 25, 27, 29, 31, 32. Monospecific antisera are, of course, required also. The pneumococcus to be typed is tested with each pool and then with the component antisera of the pool with which it reacts. Thus a pneumococcus may be identified in twelve or less tests.¹⁹

The sputum may be used directly in the *Quellung* reaction if there are sufficient pneumococci present, or it may be heated and centrifuged and the supernatant used as antigen in a precipitin-ring test (Krumwiede's method). In the latter instance the reaction is dependent upon the presence of SSS in the sputum, and a negative reaction has no significance because of the possible lack of sufficient SSS.

The intraperitoneal inoculation of white mice with sputum results in a

¹⁷ Methods of typing are discussed in detail on pp. 620-640 of Heffron: *Pneumonia*. The Commonwealth Fund, New York, 1939.

¹⁸ Kempf and Nungester. *Jour. Inf. Dis.*, 1942, 71:50.

¹⁹ For pools used in typing all 75 types see Eddy. *Pub. Health Repts.*, 1944, 59:1041.

CHAPTER 62

GLANDERS AND MELIOIDOSIS

GLANDERS

NUMEROUS investigators during the nineteenth century brought evidence to show that glanders was infectious, and that the disease in man was the same as that in the horse. The causative agent, however, was not isolated till 1882, when Loeffler and Schütz (Loeffler 1886) succeeded in cultivating *Pf. mallei* from a horse dying of acute glanders. They isolated it in pure culture from the liver and spleen, and produced characteristic lesions in the guinea-pig, rabbit, and field mouse by injection of the bacilli. The organisms were demonstrated histologically in the lesions, and isolated in pure culture.

Epidemiology in Animals.—Glanders is primarily a disease of equine animals. Pigs and cattle are absolutely resistant. Goats, sheep, dogs, and cats sometimes contract the disease naturally; it has also occurred in zoological gardens amongst carnivora, such as lions and tigers, which have been fed on infected horse-flesh.

There are two clinical types of the disease in horses and asses—glanders and farcy. In glanders, which may be acute or chronic, the lungs are almost invariably affected. Rounded, greyish, firm nodules, about $\frac{1}{2}$ to 1 cm. in diameter, appear in small numbers—often not more than a dozen or so. They are embedded in the lung tissue, from which they are not easy to enucleate. When recent they have a dirty white centre and a dark red, or sometimes yellow, gelatinous periphery; the central part consists of thick pus. In older nodules the greyish central zone is surrounded by dryish crumbling material, or by a fibrous capsule. In acute cases there may be an actual pneumonic infiltration of the lung. Histologically the young lesions consist of polymorphonuclear cells, surrounded by a zone of congestion. It is stated that, in the older nodules, there is frequently a zone of epithelioid and giant cells around the central necrotic area, and surrounding the whole there is a layer of fibrous tissue. Occasionally calcification may occur (Riegler 1905, Reinhardt 1919). Glanders may invade other organs as well as the lungs, particularly the nasal mucosa and trachea; nodules first appear, which later ulcerate and discharge a greenish-yellow serous fluid, sometimes streaked with blood (Hunting 1908, Hutyra and Marek 1926). The submaxillary glands are often enlarged; subcutaneous abscesses are not uncommon; and in the acute disease nodules are distributed throughout the spleen, liver, and other organs.

In the mule glanders is almost invariably acute, death occurring in 3 or 4 weeks (Mason 1918).

Farcy may result from direct infection of the skin, or it may be a secondary manifestation of glanders. Swellings appear in the skin or subcutaneous tissue, particularly of the extremities and flanks, which break down and ulcerate. The

rapid growth of pneumococci, and peritoneal exudate aspirated after three to six hours contains large numbers of bacteria as well as considerable amounts of SSS. The bacteria may be typed by the *Quellung* reaction, by microscopic slide agglutination (Sabin's method), or by macroscopic agglutination or precipitin tests. When mice are not available the sputum may be cultured in glucose blood broth (Avery's method) and the broth and contained pneumococci used as antigen in the immunological tests.

Variation. The smooth and rough variants that have been found in a variety of bacteria may also be observed in the pneumococcus. As in other cases, there are various intermediate colonial types between the two extremes, and the pneumococcus is virulent in the smooth form and almost completely avirulent in the rough form. The change from smooth to rough is reflected in the microscopic morphology of the cells as a loss of capsule and the ability to form SSS. It was first observed by Stryker²⁰ that pneumococci cultivated in homologous immune serum did not form capsules; the ability of specific antibody to inhibit the elaboration of capsular substance and thereby render them susceptible to phagocytosis is no doubt an important consequence of the immune response. Since type specificity is determined by the SSS, it follows that the change from smooth to rough is accompanied by a complete loss of type specificity; the somatic antigen is predominant and, irrespective of original type, the pneumococci become immunologically identical. The dissociative change may be reversed, although with difficulty, by animal passage or by cultivating the R form in the presence of anti-R immune serum, or in the presence of heat killed cells from a smooth culture.

Transformation of Types. Of fundamental biological significance, however, was the discovery of Griffith²¹ that the inoculation of mice with living R culture mixed with a heat-killed suspension of smooth pneumococci of a type other than that from which the R culture was derived resulted in the conversion of the R variant to the S variety of the new type. These transformations of pneumococcus type have also been brought about *in vitro* by cultivation of R variants in blood broth in the presence of anti-R immune serum and heat-killed smooth pneumococci of heterologous type. The S-R variation occurs in nature (its practical importance to human pneumococcus infections is not clear) and R variants may be isolated from pneumococcus infections in man, but whether the transformation of types likewise occurs in nature is not known. The transforming substance has been shown to be a polymerized ribonucleic acid which is active in inducing type transformation in very small amounts²² (see p. 182).

Pathogenicity for Man. As indicated above, lobar pneumonia is the most important pneumococcal infection in man. The bacteria are not confined to the lung, however, for they may migrate from this seat of infection through the nasal passages or be distributed via the vascular system to various parts of the body, to give rise to localized foci of infection. Pneumococcemia is of frequent occurrence; available data indicate that 50 per cent is a fair estimate. A number of workers emphasize the prognostic value of blood culture, and in

²⁰ Stryker. Jour. Exp. Med., 1916, 24.49.

²¹ Griffith. Jour. Hyg., 1928, 27.113.

²² McCarty. Bact. Rev., 1946, 10.63.

first 5 years of this century, for example, about 75 per cent. of all cases reported in Great Britain occurred in London (M'Fadyean 1905). The disease used to be especially prevalent in the large studs of horses owned by the Municipal Omnibus Companies; once the infection had been introduced, it spread rapidly throughout the stud. Apart from clinical cases there was an even larger number of carriers, which served to propagate the disease and to render its control extremely difficult.

Mode of Infection.—The mode of infection is not at all clear. Though primary disease of the lung appears to be very common, the belief has grown up that infection does not occur by the respiratory tract. It is difficult to gather from the literature the real incidence of primary glanders of the lung. There are many other diseases that give rise to lesions closely resembling the glanders nodule (Riegler 1905, Joest 1915); and our impression is that in the past many true cases of glanders have been classified under other headings and *vice versa*. Experimentally, most attempts to produce chronic pulmonary glanders *via* the respiratory tract have failed; intratracheal and intranasal injection of the bacilli have resulted in the acute disease. Most workers have therefore concluded that infection does not ordinarily occur by this route. On the other hand, typical pulmonary lesions have followed infection by the mouth (M'Fadyean 1904, Bonome 1906). In animals infected in this way, however, it is usual to find some degree of inflammatory reaction in the intestinal mucosa and submucosa, together with hyperplasia and necrosis of the mesenteric glands. As these lesions are uncommon in horses dying naturally, it is by no means certain that the disease set up in this way corresponds to that occurring spontaneously. Bonome (1906) tries to explain the discrepancy by assuming that the intestinal and mesenteric glandular lesions rapidly retrogress so that in about 2 months only the pulmonary lesions are apparent.

Without discussing this question any further, we may point to the close analogy with tuberculosis, in which much the same arguments have been advanced for and against the respiratory route of infection (see Chapter 59). Whether the infectious material in glanders reaches the lungs by direct inspiration, by absorption through the pharyngeal mucosa, or by passage through the intestine and mesenteric glands, it is impossible to say with certainty. The important point is that the nasal and pulmonary discharges, and sometimes the urine and faeces, of animals suffering from glanders are infectious, and that any other susceptible animal coming into contact directly or indirectly with these discharges is liable to contract the disease.

Reproduction of the Disease.—Farcy may be transmitted by subcutaneous inoculation. Under natural conditions, it is probable that some cases occur by direct infection of the skin—as in grooming—whereas others are really metastatic infections of pulmonary origin. In nearly every case of farcy, glanders nodules are present in the lungs at necropsy (M'Fadyean 1904).

Glanders may be reproduced in horses, asses, and mules by feeding with cultures of the bacillus, and by subcutaneous inoculation. Sheep and goats are easily infected, but cows and pigs are absolutely resistant. Of laboratory animals the hamster, the guinea-pig and the field mouse (*Arvicola arvalis*) are the most susceptible to experimental inoculation; rabbits and dogs are less so; rats, birds, and perhaps white mice, are comparatively resistant (see Chapter 19).

Epidemiology in Man.—Glanders in man is uncommon. Robins in 1906 succeeded in collecting reports of 156 cases. It is a protean disease, and probably

most instances the case fatality is considerably higher when pneumococci are present in the blood stream. Avery, Chickering, Cole and Dochez²³ observed a case fatality of 55.8 per cent in those with positive blood cultures and 8.3 per cent of patients with negative culture. Similar results have been reported by others.

Among the pathologic processes that occur as complications and sequelae of pneumococcus pneumonia, or, it may be noted, as independent and primary affections, are inflammations of the pleura, pericardium and meninges. Meningitis and otitis media are frequently secondary to pneumonia, and the connec-

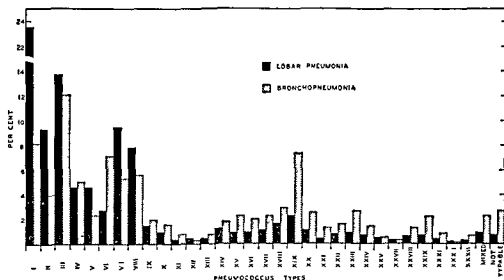


Fig 54. The incidence of pneumococcus types in lobar and bronchopneumonia observed in a two-year survey in California, Colorado, Illinois, Louisiana, New Jersey and Missouri in which pneumococci from 12,447 cases of lobar pneumonia and 3847 of bronchopneumonia were typed. The relative predominance of Type III over Type II is sometimes observed. Note the more common occurrence of higher types in bronchopneumonia, the relative incidence of mixed types, *i.e.*, those reacting to more than one antiserum, and the incidence of types other than the first thirty three. Data from Rumreich *et al.* Pub Health Repts., 1943, 58 121.

tion between inflammation of the middle ear and meningeal infection has often been noted. To those inflammatory conditions of pneumococcal origin might be added a long list of others provoked by the same organism. There appear to be few, if any, organs or tissues that are not under some circumstances subject to attack. Sinusitis, parotitis, conjunctivitis, enteritis and a great variety of other affections are occasionally due to the activity of pneumococci. In general, pneumococcus infections of this type tend to a more favorable outcome than similar infections with streptococci or staphylococci.

Resistance to pneumococcal infection is, in large part, a matter of individual predisposition, and the mere presence of pneumococci in the upper respiratory tract is not alone sufficient to bring about pulmonary infection. Specific immunity plays a negligible part in resistance, but the complex of factors as-

²³ Avery, Chickering, Cole and Dochez: *Acute Lobar Pneumonia*. Monographs of the Rockefeller Institute for Medical Research No. 7, 1917.

to agar containing 1/200,000 crystal violet so as to suppress Gram-positive organisms; colonies of the glanders bacillus may be detected by McLeod's oxidase reaction and confirmed by slide agglutination (Miller *et al.* 1948). A more sensitive method, however, is to inject the material, preferably after preliminary incubation with penicillin, subcutaneously into a guinea-pig or hamster, recover the bacillus from the enlarged glands, and inject it intraperitoneally into fresh animals. Cultures should always be tested for virulence, and the characteristic Straus reaction elicited (see p. 579). Not all strains of *Pf. mallei* are virulent on isolation; sometimes two or three passages have to be made before the typical disease develops (Dudgeon *et al.* 1918). As a rule material from the acute disease in horses proves virulent, but from the chronic disease it is generally avirulent.

The Straus reaction is not diagnostic of glanders; swelling of the testicles and periorchitis may occur after injection of guinea-pigs with organisms of the *Brucella* group, with *Actinobacillus lignieresii*, with Preisz-Nocard's *C. ovis*, with *Pf. whitmorei*, and with a few other species less commonly met with.

Mallein Test.—This is used for the diagnosis of latent or chronic glanders in horses. Mallein is prepared by growing the glanders bacillus in flasks of peptone veal broth containing 4 per cent. of glycerol. After 15 to 20 days' incubation the cultures are sterilized in the autoclave, concentrated to one-tenth of their volume in a water-bath, and filtered through paper (Nocard 1895). The resultant product is a syrupy brown liquid; before injection, this crude mallein has to be diluted about 1/10 with sterile water or 0.5 per cent. phenolized saline. Sometimes mallein is prepared by simple filtration, without concentration, of a broth culture; 1 ml. of this product is used for injection. The subcutaneous test is made by injecting 2.5 ml. of the diluted mallein under the skin of the neck. A positive reaction is characterized by (1) a local swelling, congestion, and oedema, often very extensive and painful, appearing some hours after the inoculation, increasing for 30 to 36 hours, and lasting for a week or more; it never suppurates; (2) a systemic disturbance, with a rise of temperature, dullness, depression, shivering, anorexia and other symptoms of illness; the temperature rises about the 8th hour, reaches its maximum from the 10th to the 18th hour, and subsides after 36 or 48 hours. The interpretation of the reaction is largely a matter of experience. Generally speaking, a good local reaction with a rise of temperature of 2° C. or over can be considered positive. A rise of only 1° to 2° C. may be accounted positive if the local reaction is typical; otherwise it should be considered doubtful. If the rise of temperature is less than 1.0° C., the reaction is negative. A rapid rise of temperature, however high, if succeeded by an equally rapid fall, is of no importance. Old horses may fail to react. In doubtful cases the test should be repeated after 4 weeks. In normal horses the injection of mallein causes a very slight reaction; the small local oedematous tumour disappears in 24 to 30 hours.

A positive reaction, even in the absence of clinical disease, may be taken as proof that the horse is glandered. Extensive use of the test has shown that, judged by post-mortem findings, a positive result is correct in about 92 per cent., a negative in 96 per cent. of cases. (For interpretation of the reaction see Babes 1891, M'Fadyean 1893, Bonome 1894, Foth 1891a, b, Report 1915, Mason 1918, Huttyra and Marek 1926.)

The subcutaneous test is inapplicable to horses suffering from fever. It has also the disadvantage that it is sometimes followed by the appearance of anti-

sociated with a state of physiological well-being is of the greatest significance. A preliminary depression of resistance by other infections, severe or sudden exposure to cold, fatigue and other predisposing factors, is an almost invariable preliminary to pneumococcus infection. The part played by pneumococcus pneumonia in the fatal termination of many diseases, for example, is well known.

The Pathogenicity of Pneumococcus Types. The case fatality of pneumococcus pneumonia is relatively high and indicative of the pathogenicity of these bacteria once they have become established in the lungs. The pneumococcus types differ from one another in this respect; the case fatality in Type 1 infections is 25 to 30 per cent, that of Type 2 about 40 per cent, that of Type 3, 40 to 60 per cent, and that of Group IV infections perhaps 15 to 20 per cent. Because of the relatively recent differentiation of the types comprising Group IV, data on case-fatality rates for these types are very meager as yet. The frequency of occurrence of the pneumococcal types in lobar pneumonia and bronchopneumonia is given in Fig. 54. Here the ten leading types are: 1, 2, 3, 4, 5, 6, 7, 8, 14 and 19. In some series Type 2 is more frequent than Type 3 and in others, including this one, the reverse is true.

Pneumococcus Carriers. As a strict parasite, the pneumococcus is found in man rather than in his environment. Healthy carriers of these bacteria are common; 40 to 60 per cent of groups of persons examined have been found to harbor pneumococci in the upper respiratory tract. This proportion is variable, however, being relatively high during the cold months of the year and higher among groups of contacts than among non-contacts. The carrier state is not permanent but rather sporadic and intermittent; many persons may carry these bacteria for a short time, particularly while having colds and other infections of the upper respiratory tract, while others may carry them for longer periods. There is also a seasonal fluctuation in that increased numbers are found in the winter months.

The great majority of pneumococci found in carriers are the relatively less virulent types of Group IV. Types 1, 2 and 3 are much less frequently found, but an appreciable portion of the population may harbor these types at any one time (see table). In the study made by Smillie, Calderone and Onslow²⁴ almost every type of pneumococcus was encountered; many individuals carried two or more types simultaneously. The types most commonly found were 3, 7, 21, 25 and 11 in that order of frequency. It is not clear whether an appreciably effective immunity is developed by carriers.²⁵

*The Epidemiology of Pneumococcus Pneumonia.*²⁶ Pneumococci are disseminated chiefly through the secretions and discharges of the mouth and upper respiratory tract by direct contact between persons. Droplet infection (p. 231) undoubtedly plays a large part in the transmission of these micro-organisms and perhaps accounts for the seasonal incidence of the disease (Fig. 55) and the increased frequency of carriers during the cold months of the year.

While it is obvious that pneumococcus infection is always exogenous in the

²⁴ Smillie, Calderone and Onslow: Amer. Jour. Hyg., 1943, 37:156.

²⁵ Cf. Finland, Brown and Barnes: Amer. Jour. Hyg., Sec. B, 1940, 32:24.

²⁶ See the review by Finland: Medicine, 1942, 21:307.

The precipitin reaction is not trustworthy enough for practical diagnosis, probably because of the presence of non-specific nucleoprotein substances in the organisms. Sakamoto (1930), however, extracted a soluble polysaccharide from cultures of *Pf. mallei*, with which a specific precipitation test was said to be obtainable.

A number of other tests have obtained prominence during -

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... to be of particular value in the diagnosis of the disease in mules, asses, and pregnant mares, the blood of which not infrequently has a strong anticomplementary action. According to Hole and Coombs (1947) the conglutinating complement-absorption test is more sensitive than the plain complement-fixation test in the diagnosis of experimental glanders in ponies; but it has not yet been tested under field conditions.

It will be realized that a large number of tests are available for the diagnosis of glanders. In practice it is unwise to rely exclusively on any one alone. For ordinary routine work the best combination is the complement-fixation and the conjunctival mallein tests. If these give a doubtful result, the other tests may be employed.

In man the mallein test has been used so seldom that it is difficult to gain any idea of its value. Sabolotny (1926) reports a case in which it was positive. The agglutination test has to be used with care, as the serum of normal persons almost invariably contains agglutinins in a titre ranging from 1/10 to 1/320. In glanders the titre may rise to as high as 1/5,120 (Galtier 1881, Collins 1903, Dudgeon *et al.* 1918, Cravitz and Miller 1950), but on the other hand it may be extremely low. Several cases have been reported in which no agglutination occurred at all, or only up to 1/60 (Gabriélidès and Remlinger 1902, Bernstein and Carling 1909). The complement-fixation reaction is sometimes of value (Watson 1923, Sabolotny 1926). A leucocytosis is often present in acute glanders (Gabriélidès and Remlinger 1902, Bernstein and Carling 1909).

Immunity, Treatment, and Prophylaxis.

There is a considerable amount of evidence to show that horses may recover spontaneously from glanders (Nocard 1900). Only a small proportion of apparently healthy horses that give a positive mallein reaction subsequently develop clinical signs of the disease (M'Fadyean 1900). If they are carefully observed, many of them are found to lose their reaction to mallein. It must be noted, however, that the disappearance of the mallein reaction is no proof of cure (M'Fadyean 1901, Bonome 1906). Spontaneous recovery from glanders does not leave the animals with a high degree of immunity. If exposed to natural infection or experimentally inoculated with glanders material, they not infrequently again contract the disease (M'Fadyean 1900, Nocard 1900, Mohler and Eichhorn 1914).

Numerous attempts have been made to produce an active immunity to glanders. Using graded doses of *morvine*—a substance similar to mallein—Babes (1892) stated that he was able to immunize guinea-pigs. Nicolle (1906) likewise claimed to have immunized guinea-pigs (1) by repeated doses of dead bacilli; (2) by repeated injection of living organisms in very small doses; (3) by a single injection of living organisms in a non-fatal dose. But attempts to render horses immune by vaccination or injections of mallein have not been successful (M'Fadyean 1901). Mohler and Eichhorn (1914) vaccinated horses with 3 or 4 doses of dried bacilli;

last analysis, for practical purposes it is probably endogenous in a large proportion of cases. With a high carrier rate pneumococci are frequently present in the normal individual, and when resistance is reduced to a sufficiently low level they are able to set up an infection. The high incidence of pneumococci of Types 1 and 2 (Fig. 54) as contrasted with the low carrier incidence of these types, together with the occurrence of seeming epidemics of pneumococcus pneumonia on a small scale, has been regarded by some as evidence of exogenous infection. It is more probable, however, that the greater virulence of some pneumococcus types operates as a selective factor to disturb the random distribution of types in pneumococcus pneumonia. It is known, for example, that minor respiratory infections may be common or epidemic in small groups

PNEUMOCOCCUS CARRIERS*

Persons Examined				Incidence of Carriers				
	Total	Cases Found		Total†	Per Cent Incidence of Types			
		Number	Per Cent		1	2	3	Group IV
Non-contacts..	2332	1000	42.9	1027	0.5	0.9	8.4	34.2
Contacts . .	1782	977	54.8	1018	3.3	2.7	10.0	41.0

* Modified from Heffron's¹⁷ data.

† The incidence of types is greater than the incidence of carriers because in some instances more than one type was found.

such as a family. If a given type of pneumococcus of high virulence invades and spreads within the group so that a high proportion of the individuals become carriers, the operation of factors which reduce resistance in the group may result in one or more members coming down with pneumonia due to the type carried. Smillie and Jewett²⁷ observed just such a sequence of events in a group of children in a nursery. The group was invaded by a virulent Type 14 pneumococcus which caused no harm but when the individual developed an acute respiratory infection, the dormant pneumococcus spread to the middle ear, conjunctivae and lungs. Such children were sent to the hospital ward and carried the pneumococcus which spread to most of the children there. Again, the infection was activated, sometimes with very serious consequences, on the development of respiratory infection.²⁸

Other epidemiological characteristics of pneumococcus pneumonia include seasonal incidence (Fig. 55), which corresponds roughly with the carrier rate; the age incidence, characterized by high morbidity and mortality in infants and the aged; the higher incidence in males than in females, and the apparent greater susceptibility of the Negro as contrasted with the white race.

²⁷ Smillie and Jewett: Amer. Jour. Pub. Health., 1942, 32:987.

²⁸ For a detailed experimental study of epidemic pneumonia see Hodges and MacLeod: Amer. Jour. Hyg., 1946, 44:183, 193, 207, 231, 237.

The disease appears to be primarily one of rodents. At Kuala Lumpur in the Federated Malay States, Stanton and Fletcher (1925) stated that it had been present first epidemically and later in a sporadic form amongst their stock of laboratory animals during the previous 12 years. The earliest symptom in rabbits and guinea-pigs is a white milky discharge from the eyes and nose; later dyspnoea occurs, followed by death. At post-mortem in acute cases, there are few signs except yellow miliary nodules on the nasal septum. When the disease has lasted for some time, minute caseous nodules of focal necrosis are found in the lungs, spleen, and sometimes in the liver.

Cases of natural infection have been recorded in wild rats, one case in a cat, and one in a horse. In Queensland fatal cases have occurred in sheep (Cottew 1950). Rodents can be infected experimentally by feeding and by injection of the bacilli into the tissues (see Chapter 19).

How man contracts the disease is not clear. The organism can seldom be demonstrated in wild rodents. Alain, Saint-Etienne and Reynes (1919), who studied 28 human cases, noted that some of them came on when the patient was in hospital under treatment for another illness. Some cases have undoubtedly followed contamination of the skin, as in morphia addicts, but Stanton and Fletcher (1932) regard the alimentary tract as the most probable route by which the organisms gain access to the body.

Diagnosis of the disease in man during life is not always easy. The organisms can be cultivated from the blood, from abscesses and sinuses, superficial pustules, and the urine. Splenic or hepatic puncture may be useful for obtaining infective material. In one case Martin (1931) isolated the organism from the cerebrospinal fluid. Suspected material should be cultivated on glycerol agar and inoculated into guinea-pigs or hamsters. One patient tested gave a positive mallein reaction. Agglutination is not of much value, partly because most patients die before agglutinins have had time to develop, and partly because normal agglutinins to 1/80 or over are sometimes present in healthy persons. At autopsy the organisms can be readily isolated from the visceral lesions. (For general review of the disease see Stanton and Fletcher 1932, de Moor *et al* 1932, Souchard 1932, Couture 1935.)

No satisfactory treatment is yet known. Penicillin is valueless. *In vitro* both chloramphenicol and aureomycin inhibit the growth of *Pf. whittmori*, but in experimental infections in guinea-pigs aureomycin, even when started at the time of infection, proved powerless to alter the course of the disease (Cruckshank 1919). There is some slight suggestion that the sulphonamides may have a beneficial effect.

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Bacteriological Diagnosis of Pneumococcus Infections. The pneumococcus may be isolated by culture or animal inoculation from specimens such as sputum, pleural exudate, blood, spinal fluid, pus, etc. Blood agar is the medium of choice, the bacteria growing up in twenty-four hours as small colonies surrounded by a zone of green hemolysis. It is not possible to distinguish them from *a* hemolytic streptococci by colonial or microscopic morphology but differentiation may be made by the fermentation of inulin and bile solubility of the pneumococcus and its immunological reactions. Blood specimens are cultured in buffered dextrose veal infusion broth, containing 5 mg. per 100 ml. of *p*-aminobenzoic acid if the individual is undergoing sulfa drug therapy, as in the case of culture of the streptococci. A portion of

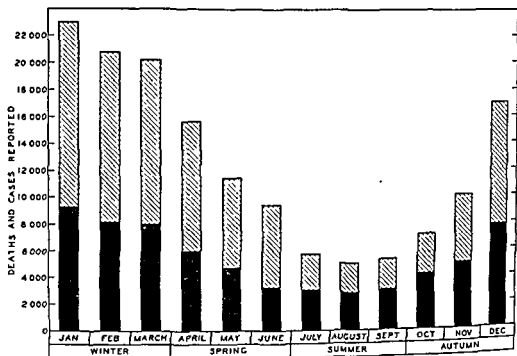


Fig. 55. The seasonal incidence of pneumococcus pneumonia. Averages of numbers of reported cases and deaths by months for the years 1939 to 1945 inclusive. Data from Supplements to Public Health Reports.

sputum, washed in three changes of sterile saline and emulsified in saline, may be inoculated intraperitoneally in a white mouse. With virulent strains the animal will show signs of illness in five to eight hours and microscopic examination of smears of peritoneal exudate will show large numbers of encapsulated diplococci.

The pneumococcus is identified and typed with antiserum, usually by the *Quellung* reaction through agglutination, precipitin tests may be used also. When large numbers of the bacteria are present in sputum, the typing may be done directly without culture or mouse inoculation, but since the *Quellung* reaction is inhibited in the presence of large amounts of SSS, negative reactions are not significant. Typing is readily carried out, as indicated earlier, on pneumococci present in mouse peritoneal exudate or cultures.

Pathogenicity for Lower Animals. The susceptibility of the usual laboratory animals to pneumococcus infection is variable, ranging from the highly

CHAPTER 63

CHOLERA

HISTORY.—Though cholera has been endemic in India for centuries, there is no record of its spread to the rest of the world previous to 1817 (Kirchner 1906). Between 1817 and 1823 it invaded many parts of Asia. The second pandemic, 1826-37, was more widespread. Starting in India, it spread to Russia in 1829, and thence to Poland, Germany, Austria, Sweden, and England. Throughout the years 1832-33 the whole of Europe was ravaged. Four thousand deaths occurred in London alone, and 7,000 in Paris. Canada and New York were infected by Irish immigrants fleeing from their native country. The population of Cuba was decimated, and there was a heavy toll of life in Mexico. The third pandemic, 1846-62, again invaded Europe and America. In 1854 the number of deaths in England was 20,000, in Italy 24,000, and in France 140,000. America was infected by way of New Orleans in 1848; thence the disease spread up the Mississippi valley and reached California. In the fourth pandemic, 1864-75, the disease prevailed widely over Asia, Africa, Europe, and America. The fifth pandemic, 1883-96, spread over Egypt, Asia Minor, and Russia; there was a severe outbreak at Hamburg in 1892; and several ports in France, Italy, and Spain were infected. The present or sixth pandemic, which started in 1902, has been confined chiefly to Asia, Egypt, and the Southern countries of Europe. In 1946 there was in China one of the largest outbreaks that has ever been experienced in the East. Though the disease has frequently been imported into England and America, since 1873 it has not succeeded in gaining a foothold. With the improved sanitary arrangements of our western civilization, cholera is being gradually restricted in its field; and it will probably not be long before it is forced back and confined once more to a few endemic foci, such as those in Lower Bengal (Craster 1913a, Elkington 1916).

Bacteriology.—At the Berlin Conference of July 1884 Koch (1886a) announced his discovery of the causative organism of cholera—the comma bacillus or *Vibrio cholerae*. During the previous year in Egypt and in India he had examined the faeces of 32 patients during life, and the intestinal contents at autopsy of 62 patients who had succumbed to the disease. In not a single instance had he failed to demonstrate the comma bacillus. The organisms were most numerous in the lower half of the small intestine. In acute cases they were present in almost pure culture, but in cases that had lasted longer, and in whom secondary changes had occurred, the vibrios were few and more difficult to find. In a smear from the rice-water stools or the intestinal contents of typical cases, the comma bacilli were arranged with their long axes parallel to one another, presenting a picture similar to that of fish in a stream. The organisms were found in the intestinal contents, in the

susceptible mouse and rabbit through the less sensitive guinea pig to the cat, dog, chicken and pigeon, which are highly resistant. Rare instances of naturally occurring infections in the usual experimental animals have been reported, such a spontaneous epizootic of type 19 pneumococcus infection in the guinea pig was reported by Homburger *et al.*²⁹ Animal experiments with the pneumococcus present an example of the general law that susceptibility is characterized by general septicemic infection, resistance by the occurrence of a localized process. The mouse and the rabbit develop a rapidly fatal septicemia, and in these animals lung lesions, when they occur at all, are slight and usually limited to the bronchopneumonic type. It is possible to produce typical lobar pneumonia in the rabbit by carefully balancing the susceptibility of the animal and the virulence of the bacterium through the use of attenuated cultures or previous partial immunization. Resistant animals, such as the dog, show an approximation toward the type of pneumococcus infection observed in man, and lobar pneumonia may be produced in monkeys by intratracheal inoculation.³⁰ The lesions produced in monkeys were considered identical with those in human lobar pneumonia. It is of some interest that pneumococci were found in the blood within six hours after their introduction into the trachea and before the signs of pneumonia appeared, suggesting the bronchogenic rather than the hematogenous origin of the infection.

Man, therefore, may be regarded as an animal of rather high normal resistance. This resistance may, however, be so reduced as to permit the production of localized manifestations, which in still more susceptible individuals may lead to a fatal septicemia. In some cases death is due to overwhelming interference with respiration caused by the local pulmonary lesions, in others, to a general systemic poisoning or toxemia.

Immunity. Experimental animals may be actively immunized against pneumococcus infection by the injection of vaccines of the smooth, virulent bacteria, although the immunity is not of long duration, *i.e.*, not more than a few months. The development of the immune state is accompanied by the appearance of antibodies, precipitins, agglutinins and the like, as well as a protective quality in the blood serum. In man the situation is somewhat obscure, there is undoubtedly an intimate relation between recovery and the appearance of humoral antibodies, but immunity following infection is slight and transitory, and one attack may succeed another after a short interval. Active immunity in experimental animals is type-specific, however, and it is not improbable that under natural conditions different immunological types may participate in successive attacks.

The question of active immunization of man to pneumococcus infection is of continued interest. Results suggestive of its value were obtained from mass inoculation studies³¹ in 1918-1919 in this country, and from the extensive studies of Felton and his co-workers.³² Heidelberger *et al.*³³ have shown that the inoculation of type-specific polysaccharides induces an antibody

²⁹ Homburger, Wilcox, Barnes and Finland. *Science*, 1945, 102: 449.

³⁰ Blake and Cecil. *Jour. Exp. Med.*, 1920, 31: 403.

³¹ Cecil. *Medicine*, 1925, 4: 395.

³² Cf. Felton *et al.*: *Pub. Health Repts.*, 1941, 56: 1041 *et ante*.

³³ Heidelberger, MacLeod, Kaiser and Robinson. *Jour. Exp. Med.*, 1946, 83: 303.

Venkatraman, Krishnaswami, and Ramakrishnan 1941) are of the opinion that the *El Tor vibrio* is not a cause of true cholera. De Moor (1949) points out that the disease—paracholera—caused by the *El Tor vibrio* is endemic, not epidemic, occurs at all times of the year, causes sporadic cases only, has a low incidence rate, and does not call for the imposition of quarantine, strict isolation of patients or mass immunization.

Pathologically, cholera is essentially a local disease confined to the intestine. The organisms multiply in the lumen and reach enormous numbers. On their autolysis they liberate toxic bodies that are apparently responsible for the irritating effect on the intestinal tract leading to severe and repeated purging, exhaustion, and death. The violence of the gastro-intestinal symptoms seems to be quite out of proportion to the almost normal appearance of the intestine at post-mortem. The organisms do not invade the blood stream, but may often be isolated from the mesenteric lymph nodes after death (Chatterjee 1939). The cholera vibrio is very susceptible to acid, and it has been suggested that lowered gastric acidity may predispose to infection; this might help to explain the higher incidence of the disease in young children and in old people, and in patients suffering from malaria, pellagra, bilharziasis, and other depressing illnesses (Abdou 1948).

Experimental Reproduction of the Disease in Animals and Man.—Natural cholera in animals is unknown, but there are certain experimental procedures by which a disease closely resembling cholera can be reproduced in guinea-pigs and rabbits.

Using dogs and rabbits, Nicati and Rietsch (1884) found that the direct injection of comma bacilli into the duodenum was followed by a fatal disease, characterized by the presence of vibrios in large numbers in the small intestine. Koch (1886b) confirmed this observation on guinea-pigs, but pointed out that the frequency of a fatal termination depended on the amount of trauma accompanying the injection. He tried to infect the animals by the mouth; and in order to enable the vibrios to pass through the stomach unharmed, he rendered the gastric contents alkaline by the administration of 5 ml. of 5 per cent. sodium carbonate solution. Twenty guinea-pigs treated in this way were shortly afterwards fed with 10 ml. of a broth culture of cholera. Only one died—an animal that had recently aborted. This suggested to Koch that the relaxation of the animal's intestine might have been responsible for the infection. Into his next batch of 35 guinea-pigs, therefore, besides giving the sodium carbonate and the cholera culture, he injected 1 ml. of *Tinct. opii* intraperitoneally to paralyse the gut, and to prolong the stay of the vibrios in the small intestine. The result was that 30 of the animals died, displaying before death weakness of the hind limbs and collapse. At necropsy, the small intestine was deeply injected and filled with a flocculent colourless fluid, containing the vibrios in almost pure culture.

response which reaches a peak within six weeks and persists for about six months; booster inoculation during the following eighteen months was not effective in raising the diminishing titers. MacLeod *et al.*³⁴ found that such immunization significantly reduced both carrier rate and cases of infection with the pneumococcus types immunized against, though not the incidence of other types. In the aggregate, then, these data suggest that an appreciable degree of effective immunity may be produced by immunization procedures.

The results of the therapeutic use of antiserum are variable, being excellent with some types of pneumococci and not with others. The treatment of Type 1 infections with specific antiserum is relatively successful in that the case fatality is reduced from 25 to 30 per cent to 12 per cent. Type 2 infections do not respond quite so well to serum therapy, the case fatality being reduced from about 40 per cent to 25 per cent, and Type 3 infections are little benefited. Of the types comprising Group IV, Types 5 and 7 appear, on the basis of present evidence, to respond to serum therapy. Specific serum therapy is complicated by the existence of the multiplicity of higher types of pneumococci. Diagnostic and therapeutic antisera have been made available commercially for thirty-two types of pneumococci, but it seems probable that polyvalent sera, based on the frequency of occurrence of the higher types, will become a practical necessity.³⁵ Combinations to give such polyvalent antisera have been suggested by Eddy.³⁶

Sulfonamide and penicillin therapy has, to a large extent, supplanted serum therapy. Antiserum is generally prepared in horses, but some observations³⁷ have suggested that rabbit antisera may be more effective, in part because higher protective titers are obtainable than with horse serum and in part because the smaller antibody molecules (p. 309) should facilitate absorption. Pneumococcus antisera are standardized by titration of mouse protective antibody.³⁸

It may be noted here that a bacterial enzyme decomposing the Type 3 specific polysaccharide isolated by Avery and Dubos³⁹ protects experimental animals against Type 3 pneumococcus infection.⁴⁰ The injection of the enzyme preparations, however, produces untoward reactions, notably a febrile reaction and leucopenia, and therefore they have not been used in man.

³⁴ MacLeod, Hodges, Heidelberger and Bernhard: *Jour. Exp. Med.*, 1945, 82:445.

³⁵ See the comprehensive review of this matter by Finland: *Jour. Amer. Med. Assn.*, 1942, 120:1294.

³⁶ Eddy: *Pub. Health Repts.*, 1944, 59:1485.

³⁷ Horsfall, Goodner and MacLeod: *Science*, 1936, 84:579; *Jour. Amer. Med. Assn.*, 1937, 108:1483, Goodner, Horsfall and Dubos: *Jour. Immunol.*, 1937, 33:279.

³⁸ Cf. Military Surgeon, 1944, 94:386.

³⁹ Avery and Dubos: *Science*, 1930, 72:151.

⁴⁰ Avery and Dubos: *Jour. Exp. Med.*, 1931, 54:73, Goodner, Dubos and Avery: *Jour. Exp. Med.*, 1932, 55:393.

many of the problems presented by the disease. From a careful study of case histories he brought strong evidence to show that infection spread from one person to another. He concluded that the poison was of particulate nature, that it was swallowed accidentally, that it increased in the stomach and bowels, and that it was voided in the stools. He gave the first properly documented description of a water-borne outbreak—the famous outbreak associated with the Broad Street pump in Golden Square; and by a further painstaking study of the distribution of cholera cases among inhabitants in South London supplied by different water undertakings brought convincing evidence of the part played in the dissemination of the disease by water specifically contaminated with human excrement. He noted that soiled linen might spread the infection and that flies might carry the poison mechanically; and he anticipated current practice by his observation that both storage and sedimentation of water led to the disappearance or decomposition of the poison. Finally he laid down rational preventive measures based on personal cleanliness, avoidance of the faecal contamination of food and drink, and destruction of the poison by cooking or other means.

In India cholera is most prevalent during the hot weather. Thus in Lower Bengal the maximum incidence is reached from February to May; in the Punjab from May to October (Rogers 1911). The disease is commoner in men than in women. The case fatality is lowest in the 11-20 age group, and increases with age; in the figures cited by Rogers the case fatality in patients between 11 and 20 years of age was 51.3 per cent., in those over 50 years of age it was 73.7 per cent. The effect of atmospheric conditions seems to vary in different parts of India; those who are interested will do well to study the very careful statistical analyses made by Russell and Sundararajan (1928). The disease in India occurs with a certain periodicity, and by calculations based on the relevant data it is said to be possible to predict an epidemic 2 or 3 months ahead of its occurrence (Russell and Sundararajan 1926-27).

The true home of cholera is the delta of the Ganges. Here the disease has occurred year after year for centuries. Previous to 1817 it had apparently never spread widely from this area, but since that year it has manifested an increasing disposition to invade not only other parts of India, but almost the whole world. The only other major endemic area is the Yangtse valley. The disease appears to be endemic in Central and Southern Madras, Burma and the Philippines. It recurs frequently in other parts of China, in Indo-China, Thailand, the Dutch East Indies, the Philippines, and Japan, but these should probably not be regarded as true endemic centres (Bernard 1935). In India the number of deaths from cholera during the 10 years 1898-1907 was 370,000 or 1.64 per 1,000 of the population. It is the low-lying alluvial tracts and the seaports that are most invaded; on high land the disease spreads less readily.

Mode of Spread.—There are many ways in which infection can be transmitted from the sick to the healthy person. A study of the epidemiology of cholera indicates, however, that outbreaks can be divided into those dependent on (1) water-borne infection, and (2) case or carrier infection.

We have already referred to the classical water-borne outbreak in 1851 described by John Snow. Another example is the Hamburg epidemic of 1892. Hamburg, and two of its suburbs—Altona and Wandsbeck—were provided each with a different water supply. Hamburg drew its water from the river Elbe at a point above the city, and did not filter it. Altona drew its water from the Elbe below

Chapter 17

THE GRAM-NEGATIVE PATHOGENIC COCCI (NEISSERIA): THE GONOCOCCUS AND THE MENINGOCOCCUS

The gonococcus and the meningococcus are the chief representatives of a small group of closely related bacteria whose other members are nonpathogenic inhabitants of the mouth and upper respiratory tract of man. Two groups of species are separated from one another on the basis of pigment production, and further differentiation is made by means of fermentation reactions.

FERMENTATION REACTIONS OF THE GRAM-NEGATIVE DIPLOCOCCI

Non-pigmented Species	Dextrose	Maltose	Sucrose	Levulose	Mannitol
<i>N. gonorrhoeae</i> (gonococcus)	+	-	-	-	-
<i>N. intracellularis</i> (meningococcus),	+	+	-	-	-
<i>N. catarrhalis</i>	-	-	-	-	-
<i>N. sicca</i>	+	+	+	+	-
Pigmented Species					
<i>N. perflava</i> (flava I) . .	+	+	+	+	+
<i>N. flava</i> (flava II)	+	+	-	+	-
<i>N. subflava</i> (flava III)	+	+	-	-	-
<i>N. flavescens</i>	-	-	-	-	-

Pigmented varieties are frequently found in the nasopharynx (*Neisseria flava* I, II, III).

THE GONOCOCCUS¹

Neisser² in 1879 first called attention to the constant presence of a peculiar coccus in gonorrhoeal pus. In cases of gonorrhoea of recent origin this was the sole organism found, it not only occurred in the urethral and vaginal discharges of ordinary gonorrhoea, but was present in the exudate in conjunctivitis

¹ Present knowledge of gonorrhoea is reviewed in the report of the Committee for Survey of Research on the Gonococcus and Gonococcal Infection, Thomas and Barney-Jones, Amer. Jour. Syph., 1936, 22 Suppl. to No. 1.

² Neisser, Centralbl. f. d. med. Wissensch., 1879, 17:497.

dead in a week; none survived longer than 2 weeks. In Nile water contaminated with faeces the vibrio survived for 4 days (Gohar and Makkawi 1948). Some natural waters, for example the Jumna and the Ganges, appear to be unfavourable to its survival (Hankin 1896).

Speaking generally, we may say that heat and desiccation are rapidly destructive, but that under suitable conditions of moisture and temperature the cholera vibrio may survive outside the body for a sufficient length of time to be of epidemiological importance.

Patients who have recovered from cholera usually cease to excrete the organism within a few days. Gilmour (1952), who observed 113 cholera patients in Calcutta until 5 or more successive daily negative cultures were obtained from the stools, found that approximately 70 per cent. were negative in one week after the onset of the disease, 90 per cent. in two weeks, and 98 per cent. in three weeks. Four patients excreted the vibrio intermittently for 20, 21, 23 and 25 days respectively. Longer periods of excretion have been recorded but, as Gilmour points out, cross-infection and reinfection cannot always be excluded. Some of the older records suffer from the fact that the identification of the vibrio was not as exact as it is now.

In Shanghai, Peterson (1946) found no persistent carriers; 99.8 per cent. of the 1949 patients he examined were negative by the end of the second week, and only one patient excreted the vibrios for as long as 17 days. It is probable that the vibrio often survives in the gall-bladder and from there is excreted into the intestine. Greig (1913) isolated it from the bile of 30 per cent. of fatal cases of cholera and Chatterjee (1939) from 60 per cent. (see also Elkington 1916). Its isolation has been reported from the urine (Greig 1913-14a).

Healthy contacts may become infected and excrete the vibrios without manifesting any sign of the disease. In endemic areas in India where "cholera nests" exist, Read and Pandit (1941) isolated the organism from the stools of 7 per cent. of close contacts of cholera cases. Occasionally cholera develops in these healthy carriers—so-called precocious carriers—and, if this occurs some time after their contact with cholera patients, it may be very puzzling to trace the source of their infection. Munson (1915), for example, discovered three healthy carriers in a prison in Manila. These were isolated and examined daily. Two of them, after having been carriers for 16 and 17 days respectively, developed cholera, and the third developed it after 18 days and died in 8 hours. As Munson points out, this man might have travelled half-way round the world scattering his infection broadcast during his 18-day period as a carrier, and died of true cholera in a place many thousands of miles from any other source of infection. These cases are probably exceptional, but there is evidence to suggest that many persons do not develop the disease clinically for some hours—24 or more—after the vibrios are demonstrable in the faeces (Crendiropoulo 1912). Possibly the Egyptian epidemic of 1917, which started at El Korein (Shousha 1948), may have been initiated by a carrier from abroad, but its origin remains obscure.

The spread of contact infection may be aided to some extent by flies. Cholera vibrios have been isolated from flies taken in infected houses, from flies caught in a post-mortem room in which cholera corpses had been examined, and from the feet of flies caught 17 hours after their experimental contamination (Elkington 1916).

Contact infection is responsible for the usual *chain-spread* of cholera. Cases

due to gonorrheal infection. Pure cultures of this microorganism were isolated in 1885 by Bumm,³ who succeeded in demonstrating its etiologic relation to gonorrhea by the inoculation of human volunteers. This bacterium, known generally as the *gonococcus*, has been termed *Micrococcus gonorrheae* and *Diplococcus gonorrheae*, but the genus *Neisseria* is now more or less generally accepted and this bacterium is properly known as *Neisseria gonorrheae*.

Morphology and Staining. In preparations made from gonorrheal pus the cells of the gonococcus occur in pairs, with the flattened sides in juxtaposition, the appearance in stained preparations resembles that of a coffee bean. In pure culture the cocci appear as oval or spherical and are often aggregated in irregular masses without the typical diplococcus arrangement. In pus smears the gonococcus occurs almost entirely within the leucocytes; frequently enormous numbers may be found packed within a single phagocyte. In the earliest stages of infection, however, gonococci may be found extracellularly, and the



Fig. 56. The gonococcus from pure culture. Fuchsin; $\times 1050$.

same is true of cases of gonorrhea of long standing. The gonococcus is non-motile and does not form spores.

The colonies of the gonococcus are small, translucent, finely granular with lobate margin and grayish white with a pearly opalescence when viewed by transmitted light. Larger colonies may be formed on special media. Colonial appearance is, however, subject to considerable variation (see below).

Unlike the pyogenic cocci, the gonococcus and related forms are gram-negative, a staining characteristic that is of considerable diagnostic value since it serves to differentiate the gonococcus from other cocci present in the urethral or urovaginal tracts. Other gram negative cocci may be found occasionally, sometimes within the leucocytes, but they are rare. The tendency to decolorization in the gram stain is variable. Some strains decolorize much more readily than others, and gonococci embedded in masses of pus may retain the stain; hence the preparation of thin, uniform films is highly desirable. The

³ Bumm. *Der Mikroorganismus der gonorrhoeischen Schleimhauterkrankungen* Wiesbaden. 1885.

off, they leave no stain. On deoxycholate citrate medium the colonies appear as non-lactose fermenters, more translucent than salmonella colonies and generally smaller.

Colonies should be thoroughly rubbed up in saline so as to form a homogeneous suspension, and tested by slide agglutination against a suitable dilution of a serum containing antibodies to both the O antigens of Gardner and Venkatraman's sub-group I (see p. 609). A provisional identification may be made by this means, but it should be remembered that one of the O antigens of *V. cholera* is shared with *Bact. faecalis alkaligenes* and an H factor with *Salmonella enteritidis* (see Gohar and Makkawi 1918). The slide test, if positive, should be confirmed by the tube method, and the colony should be inoculated into peptone water for the cholera red test, into mannose sucrose and arabinose, into glucose phosphate medium, and into Douglas' broth for haemolysin production (see Chapter 22). A vibrio giving a positive cholera red and a negative Voges-Proskauer reaction, failing to form a soluble haemolysin to sheep or goat cells, fermenting mannose and sucrose but not arabinose, and agglutinable by a specific O antiserum may be regarded as a cholera vibrio. If further confirmation is required, it may be tested for pathogenicity by the intraperitoneal inoculation of mice or young guinea-pigs, and for susceptibility to lysis by a high-titre cholera antiserum—Pfeiffer's (1895) reaction. Vibrios having the O antigen of sub-group I, but haemolysing sheep or goat cells, belong to the El Tor group.

In typical cases of cholera direct plating on to a suitable solid medium is the most expeditious and reliable method of diagnosis. In other cases and in carriers a fluid enrichment medium, such as Read's (1939) modification of Wilton and Blair's medium, should be used in addition. Several methods have been employed for the isolation of cholera vibrios from water; enrichment media are required, and an attempt may be made to concentrate the organisms by preliminary filtration through a porcelain candle (see Panja and Ghosh 1947). Cholera vibrios from acute cases are in the smooth phase, but from convalescents and from water they may be partly or wholly rough and magglutinable by smooth O antiserum.

During an epidemic of cholera the bacteriological diagnosis can usually be made by simple microscopical and cultural examination, supplemented, if necessary, by the cholera-red and the agglutination reaction. But in non-epidemic times, or at the beginning of an epidemic, especially in a country usually free from cholera, the diagnosis should never be made till all the tests described have been performed and found positive.

The identification of vibrios from carriers or cholera contacts, and from non-human sources such as water or milk, is often very difficult, and has to be performed with the utmost care. Vibrios are often found under these conditions (Dunbar 1893, Kutscher 1893, 1895, Gotschlich 1895, 1906, Haffkine and Simpson 1895, Neufeld and Haendel 1907, Ruffer 1907, Zlatogoroff 1909, Crendiropoulos 1912, Cranster 1913a, b, 1914, Jermoljew 1926) that bear an extremely close resemblance to the cholera vibrio, and can be distinguished from it only by intensive study. Even in cholera stools, antigenically atypical forms, such as those described by Chen (1932-33), Yang and White (1934), Aoki and Oshiro (1931), and White (1935), are often found. In the examination of such vibrios, attempts should be made by repeated subculture and intraperitoneal inoculation of mice or guinea-pigs to bring about a change to the typical antigenic form. Most non-cholera vibrios are harmless to guinea-pigs, but the pathogenicity test cannot be relied on exclusively.

In the examination of suspected convalescents, besides looking for the vibrio

gonococcus stains with the aniline dyes, but polychrome stains, such as Pappenheim's stain,⁴ are more useful. Intracellular granules may be found in stained preparations, but in general the gonococci from young cultures stain evenly, while older cultures (twenty-four hours and older) contain large swollen in solution forms which may stain poorly.

Physiology. In its nutritive requirements the gonococcus is one of the most fastidious bacteria, particularly upon primary isolation. An enriched medium is, of course, required for cultivation. Earlier media were enriched by the addition of ascitic and hydrocele fluid. A proteose-3 hemoglobin agar has been used to some extent in this country, but the most satisfactory medium is chocolate (heated blood) agar prepared with an infusion base. Nutritional requirements appear to be highly complex and are not well known. Mueller and Hinton⁵ have devised a protein free hydrolyzed casein-starch medium that may be autoclaved and Boor⁶ has been able to grow the gonococcus on a medium containing tryptic digest of casein, cystine, dextrose and buffer. The cystine appears to be required by the gonococcus but not by the meningococcus. Growth is markedly stimulated by glutathione in many cases, and in others strains appear to require it. Synthetic media have been devised by Welton, Stokinger and Carpenter⁷ and by Gould, Kane and Mueller⁸ which contain amino acids, magnesium, iron, etc., and will support growth of the gonococcus. A sufficient supply of moisture is essential, there should be water of condensation on the tubes or plates and the atmosphere of the incubator should be kept saturated with water. Incubation in an atmosphere of increased CO₂ tension, about 10 per cent, greatly improves growth and is a practical necessity in primary isolation. With continued cultivation on laboratory media, the gonococcus appears to become somewhat less fastidious and some strains may eventually grow upon the ordinary infusion media. The preservation of cultures is difficult, however, for the gonococci die off in two to three days at room temperature and in six to eight days at 37° C. but will live longer when kept in the cold. Even upon continued transfer the gonococci die off and cultures are frequently lost. The optimum temperature for growth is 37° C. growth does not occur below 30° C., and temperatures of 40° to 41° C. are definitely harmful. The gonococcus will grow sparsely under anaerobic conditions but is essentially aerobic in character.

With respect to deleterious influences the gonococcus is a delicate microorganism. It is readily killed by heat as indicated above and by dilute antiseptics. 1 per cent phenol, for example, kills in one to three minutes. It is remarkably sensitive to certain of the flavine dyes (p. 149) and is rapidly destroyed by silver salts. The gonococcus is sensitive to drying and, under ordinary conditions, can survive exposure to the air for only a very short time—one to two hours—although in masses of dried pus it may live exceptionally for six to seven weeks.

The gonococcus is not very active biochemically. Glucose is fermented, prin-

⁴ A methyl green pyron or stain. For recent studies on this stain see Barnet. *Bull. Med. Jour.*, 1944, 1494.

⁵ Mueller and Hinton. *Proc. Soc. Exp. Biol. Med.*, 1941, 45:430.

⁶ Boor. *Proc. Soc. Exp. Biol. Med.*, 1942, 5:22.

⁷ Welton, Stokinger and Carpenter. *Science*, 1944, 99:372.

⁸ Gould, Kane and Mueller. *Eur. East.*, 1944, 47:287.

and the uninoculated. For this reason statistical analysis of most of Savas' figures is not likely to afford much information on the value of vaccination. An exception must be made for the Sanitary Corps, nearly every member of which received two inoculations before the disease broke out. A comparison made between the incidence of cholera among the members of the Sanitary Corps and among the combatants is recorded in Table 113 (taken from Greenwood and Yule 1915).

The value of χ^2 is high; and the probability that the difference in the attack rates between the two groups is attributable to chance is much less than 1 in 10,000 (see p. 1748). Greenwood and Yule (1915) concluded from these results that "anti-cholera inoculation . . . is a prophylactic step of importance, although an exact statistical measure of the degree of relative immunity conferred cannot be provided."

A more recent attempt to assess the protective value of vaccination against cholera was made in India by Adishesan, Pandit and Venkatraman (1947) and by Sekar (1947), who studied the incidence of the disease among inoculated and uninoculated subjects in Madras Province during 1942-8. The figures were collected during an epidemic and are therefore open to the unavoidable criticisms

TABLE 113

	Not Attacked.	Attacked.	Total.
Sanitary Corps	2,884	13	2,897
Combatants	112,613	2,192	114,805
Total	115,497	2,205	117,702

$$\chi^2 = 32.79. \quad P = \text{less than } 0.0001.$$

which this method of approach entails. Nothing, we believe, but a properly controlled trial will tell us what the real value of vaccination is.

Kolle's vaccine—a 24 hours' agar culture killed by heat at 55° C. for 1 hour and preserved with 0.5 per cent. phenol—or a modified vaccine prepared by growing the vibrio in broth for 3 days at 37° C., adding 0.05 per cent. formal, and incubating for 3 days at 37° C., is now generally used. The standardization and dosage vary in different parts of the world. Two doses, each of 4,000 million organisms, should be given at a week's interval, or if only a single dose is practicable 8,000 million. The vaccine should be prepared with strains of the Inaba and the Ogawa-Hikojima types so as to contain both O antigens of sub-group I. Other methods of vaccine preparation have been described (see Sokhey and Habbu 1950).

Bacteriophage Treatment.—Though the administration of bacteriophage prophylactically or therapeutically has been recommended by others than d'Herelle (see Morison 1932, Asheshov *et al.* 1930, Asheshov 1933), there is very little evidence to suggest that it is of any particular value. The collection of reliable figures is peculiarly difficult, and is rendered even more so by the fact that under primitive conditions of sanitation the bacteriophage is rapidly transferred from one person to another, thus stultifying the usual experimental method of treating alternate subjects. There is reason to believe that the replacement of smooth by rough colonies of the cholera vibrio in the intestine of convalescent cases is due to the

due to gonorrheal infection. Pure cultures of this microorganism were isolated in 1885 by Bumm,³ who succeeded in demonstrating its etiologic relation to gonorrhea by the inoculation of human volunteers. This bacterium, known generally as the *gonococcus*, has been termed *Micrococcus gonorrheae* and *Diplococcus gonorrheae*, but the genus *Neisseria* is now more or less generally accepted and this bacterium is properly known as *Neisseria gonorrheae*.

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³ Bumm. *Der Mikroorganismus der gonorrhoeischen Schleimhauterkrankungen*. Wiesbaden, 1885.

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Physiology. In its nutritive requirements the gonococcus is one of the most fastidious bacteria, particularly upon primary isolation. An enriched medium is, of course, required for cultivation. Earlier media were enriched by the addition of ascitic and hydrocele fluid. A proteose-3 hemoglobin agar has been used to some extent in this country, but the most satisfactory medium is chocolate (heated blood) agar prepared with an infusion base. Nutritional requirements appear to be highly complex and are not well known. Mueller and Hinton⁵ have devised a protein free hydrolyzed casein-starch medium that may be autoclaved and Boor⁶ has been able to grow the gonococcus on a medium containing tryptic digest of casein, cystine, dextrose and buffer. The cystine appears to be required by the gonococcus but not by the meningococcus. Growth is markedly stimulated by glutathione in many cases, and in others strains appear to require it. Synthetic media have been devised by Welton, Stokinger and Carpenter⁷ and by Gould, Kane and Mueller⁸ which contain amino acids, magnesium, iron, etc., and will support growth of the gonococcus. A sufficient supply of moisture is essential, there should be water of condensation on the tubes or plates and the atmosphere of the incubator should be kept saturated with water. Incubation in an atmosphere of increased CO₂ tension, about 10 per cent, greatly improves growth and is a practical necessity in primary isolation. With continued cultivation on laboratory media, the gonococcus appears to become somewhat less fastidious and some strains may eventually grow upon the ordinary infusion media. The preservation of cultures is difficult, however, for the gonococci die off in two to three days at room temperature and in six to eight days at 37° C. but will live longer when kept in the cold. Even upon continued transfer the gonococci die off and cultures are frequently lost. The optimum temperature for growth is 37° C., growth does not occur below 30° C., and temperatures of 40° to 41° C. are definitely harmful. The gonococcus will grow sparsely under anaerobic conditions but is essentially aerobic in character.

With respect to deleterious influences the gonococcus is a delicate microorganism. It is readily killed by heat as indicated above and by dilute antiseptics, 1 per cent phenol, for example, kills in one to three minutes. It is remarkably sensitive to certain of the flavyne dyes (p. 149) and is rapidly destroyed by silver salts. The gonococcus is sensitive to drying and, under ordinary conditions, can survive exposure to the air for only a very short time—one to two hours—although in masses of dried pus it may live exceptionally for six to seven weeks.

The gonococcus is not very active biochemically. Glucose is fermented, prin-

⁴ A methyl green pyronine stain. For recent studies on this stain see Barratt. *Brit. Med. Jour.*, 1944, 1494.

⁵ Mueller and Hinton. *Proc. Soc. Exp. Biol. Med.*, 1941, 45: 330.

⁶ *Proc. Soc. Exp. Biol. Med.*, 1942, 51: 22.

⁷ Welton, St. Linger and Carpenter. *Science*, 1944, 99: 372.

⁸ Gould, Kane and Mueller. *Jour. Bact.*, 1944, 47: 287.

CHAPTER 64

MENINGITIS

INTRODUCTORY

MENINGITIS is an inflammatory affection of the membranes surrounding the brain and spinal cord, which occurs sometimes as a primary disease, and sometimes secondarily to disease of some other part of the body.

Before the advent of bacteriology the tuberculous form was recognized, the cerebrospinal form when it occurred in epidemics, and the post-basic form in children.

The discovery of the tubercle bacillus by Koch in 1882 provided a bacteriological means of distinguishing the tuberculous from other forms of meningitis. Pneumococci were shown by Fraenkel (1886), Foà and Bordoni-Uffreduzzi (1886), and others to be responsible for the meningitis which occasionally complicates lobar pneumonia. In 1887 Weichselbaum of Vienna published his classical paper on the finding of a Gram-negative diplococcus in six cases of acute cerebrospinal meningitis; this organism he named the *Diplococcus intracellularis meningitidis*. The same year his work was confirmed by Goldschmidt (1887) and by Edler (1884-88). Jaeger (1895), in describing the differences between the pneumococcus and the meningococcus, assigned to the latter characters which it does not possess, and numerous papers were published during the following 10 years dealing with the differences between his description and that of Weichselbaum (Scherer 1895, von Hibler 1896, Heubner 1896, Kiefer 1896, Kister 1896, Still 1898, Councilman *et al.* 1898, Faber 1900, Jaeger 1903a, b, Albrecht and Ghon 1901, 1902, 1903). The correctness of Weichselbaum's observations was finally established by the extensive studies of von Lingelshiem (1905a, b) during an epidemic of cerebrospinal fever in 1904-5. Since that date the *Diplococcus intracellularis meningitidis* of Weichselbaum, or, as it is now called, *Neisseria meningitidis*, has been recognized as the causative organism of the disease. Still showed in 1898 that the post-basic meningitis of children is due to a Gram-negative diplococcus apparently identical with Weichselbaum's, and subsequent work has shown that the two organisms are the same.

Other forms of meningitis will be dealt with at the end of the present chapter.

Cerebrospinal Meningitis

This is an infectious disease characterized by inflammation of the meninges, particularly at the base of the brain. It may be acute or chronic, and may occur sporadically or epidemically. The causative agent is the meningococcus. The disease can be reproduced experimentally by the intrathecal inoculation of virulent meningococci into monkeys and rabbits (see Chapter 23). There is reason to

cipally to lactic acid, but many other sugars are not attacked, indol is not produced, nitrates are not reduced, and no change is produced in litmus milk. Catalase is produced, and a characteristic that has been turned to practical differential use is the formation of indophenol oxidase. McLeod *et al.* recommend the cultivation of suspected material on 10 per cent heated blood (chocolate) agar in an atmosphere containing 8 per cent carbon dioxide, followed by twenty-four hours' incubation in air. A 1 per cent solution of tetramethyl-*p*-phenylenediamine is poured on the incubated plate and poured off again immediately or sprayed on with a nasal atomizer. Colonies of bacteria forming indophenol oxidase turn a bright purple color. The bacteria are not immediately killed and may be subcultured within half an hour. This so-called

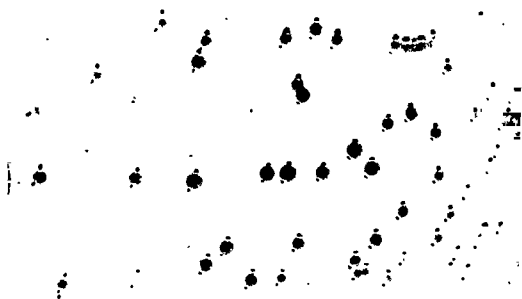


Fig. 57. Colonies of the gonococcus on blood agar. $\times 6$.

"oxidase reaction," coupled with the examination of smears for gram-negative intracellular diplococci, is becoming generally used in the laboratory diagnosis of gonorrhea.

Toxins. Although a number of attempts were made by the early workers to demonstrate the formation of toxins by the gonococcus, variable results were obtained. These bacteria form a weak hemolysin, and their cell substance will kill laboratory animals when injected in sufficient amount and will evoke suppuration upon instillation into the human urethra. A carbohydrate-lipid complex has been prepared by Boor and Miller⁹ by extraction of the cells with M/2 trichloroacetic acid which is both antigenic and toxic to mice and may be regarded as an endotoxin.

Variation. Numerous workers have observed that the cultural characteristics of the gonococcus are subject to considerable variation. It has been found¹⁰ that two types of colonies may be observed which appear to be correlated with immunologic type. The one, designated as Type I, is a large, ir-

⁹ Boor and Miller. Jour. Inf. Dis., 1944, 75:47.

¹⁰ Atkin: Brit. Jour. Exp. Path., 1925, 6:235.

cases with 2,026 deaths (Flexner 1907a). Extensive outbreaks were reported in France in 1909-10, and in Texas in 1912. More recently the disease has increased in some countries. There was a particularly severe outbreak in the United States in 1928-30 (see Hedrich 1931). In England and Wales the incidence rose from 300 cases and 284 deaths in 1923, to 2,152 cases and 1,440 deaths in 1931. Thereafter it declined, but rose sharply at the beginning of the second world war to reach in 1940 a maximum of 11,162 cases and 2,459 deaths. By 1951 both incidence and mortality had fallen by nearly 90 per cent.

The disease attacks particularly children and adolescents. Compton (1918) finds that the most susceptible age is 0 to 5 years; the susceptibility is slightly less from 5 to 10, distinctly less from 10 to 15, and for the remainder of life it remains fairly low—at about $1/5$ of that of the 0 to 5 age group. In the Danzig epidemic of 1865, 93 per cent. were under 5 years of age. The disease is unusual, however, in the first 3 months of life. It is somewhat commoner amongst males, but this appears to be related more to increased opportunity for infection than to any special sex predisposition. Miners and soldiers are the two occupational classes on which the disease falls most heavily, especially young recruits. The seasonal incidence is striking, the greater proportion of cases occurring in winter and spring; during 1915 and 1916 in England, 77 per cent. of the cases were in the first six months of the year. Much has been written about weather conditions. A low temperature, a cold wind, and increased humidity of the atmosphere appear to be predisposing agents (Dopter 1921), but it seems probable that these act mainly by leading to overcrowding indoors. Fatigue exerts a powerful influence; it is the recruits, unaccustomed to the rigours of military life, who furnish the greater number of cases in the Army. Dopfer tells an impressive story of a party of recruits who made a long, fatiguing march to join their regiment at Versailles. On reaching their destination cerebrospinal fever broke out, and of a total strength of 153 men no fewer than 79 developed the disease.

II. Mode of Spread of the Disease.—The disease is endemic in large towns, from which, at least in the post-basic form, it rarely disappears. Every winter and spring a few fresh cases occur—sporadic cases affecting children, less frequently young adults—which appear at widely separated points having no apparent connection with each other. In villages the endemic state is practically unknown.

Every now and then an epidemic breaks out. As a rule it starts insidiously by the occurrence of one or two cases at intervals of a week or more; it then begins to advance by the formation of small multiple foci in families, schools, barracks, or gaols. Often it works itself out in one focus before passing on to another. Thus, at Strassburg (Dopter 1921) in 1840 the 7th line regiment was attacked in October; three months later it spread to the 69th regiment, of which two companies were quartered with a part of the 7th. In January the 29th line regiment and the 11th artillery were attacked; in February the 34th line regiment and the 1st artillery; and in March, 6 months after the commencement of the outbreak, it invaded the pontonniers.

In a town the cases are not aggregated together, but occur widely scattered, as if there were no causal relationship between them. Thus in Hamburg between 1880 and 1885 the 180 cases of cerebrospinal fever that occurred were distributed over 131 streets.

The spread of the epidemic is irregular and capricious; often groups, which from their situation appear certain to be attacked, escape, whereas others, situated

regular, flattened, translucent colony that, on continued incubation, gives rise to surface papillae; the round, raised with slightly are generally recovered from laboratory strains and, so thought to represent the first step in transition toward the Type II form. The relation of these forms to the dissociative changes of other bacteria is uncertain. Small colony variants, presumably arising from a dissociative process, have been produced experimentally¹¹ and have also been found on primary isolation from clinical material.¹²

Classification. The relationship of the gonococcus to the other members of its genus has already been discussed and need not be considered further. The gonococci are immunologically heterogeneous, and a number of attempts have been made to subdivide them into types. Most of the freshly isolated strains from acute cases appear to fall in one serological group, while old stock cultures and strains from chronic cases constitute a second subdivision which is regarded by some as a degenerative form. "Intermediate" and serologically "independent" strains occur, and a sharp differentiation may not be made. The chemical fractionation of gonococci has indicated the presence of polysaccharide and nucleoprotein components which are shared by other species of *Neisseria*, the meningococcus and *Neisseria catarrhalis*.¹³ Type-specific carbohydrate has been found in gonococci which is said to be responsible for the immunological character of Type I and II, the two groups noted above. Specific agglutinating antisera may be prepared in rabbits and chickens by the intravenous inoculation of living gonococci. The significance of these findings to gonococcal infection is as yet uncertain.

Pathogenicity for Man. Few diseases are so widely disseminated through all classes of society as gonorrhea. Precise information as to the incidence of the disease is not available, a morbidity rate of 10 per cent in the United States is generally accepted as a conservative estimate. It is calculated that about a million fresh gonococcal infections occur each year, and there is no evidence of a downward trend.¹⁴

As a rule the gonococcus attacks primarily the human urethra and gives rise to an inflammation which may be followed by chronic urethritis and stricture. There is a marked tendency for spread of the infection along contiguous mucous surfaces, resulting, in the male, in epididymitis and other inflammatory conditions. In the female, the entire genito-urinary tract may be involved, and the fallopian tubes, the ovaries and the peritoneum are not uncommonly invaded. The gonococcus may also invade the blood stream from local lesions and be carried to various parts of the body and give rise to a variety of extra-genital lesions. Especial predilection is shown for the synovial membranes of the joints, where it causes the so-called "gonorrheal rheumatism," and for the heart valves, where it produces endocarditis. Local or general complications

¹¹ Raven Jour. Inf. Dis., 1934, 55 328.

¹² Morton and Shoemaker: Jour. Bact., 1945, 50:585.

¹³ Boor and Miller. Jour. Exp. Med., 1934, 59 63; Miller and Boor: Jour. Exp. Med., 1934, 59 75

¹⁴ Vonderlehr and Usilton: Amer. Jour. Syph., 1938, 22:537.

During the war of 1914-18 observations of much interest were made on the carrier rate. By swabbing large numbers of the military population in camps and depots, it was found that previous to an outbreak of cerebrospinal fever the proportion of carriers of the meningococcus increased steadily. The normal carrier rate amongst troops was recorded as 2-4 per cent., but preceding an epidemic it rose till it reached 20-30 per cent. Soon after it had passed the 20 per cent. limit, isolated cases of meningitis began to appear, and as the epidemic gained foothold the carrier rate likewise rose, sometimes to as high as 88 per cent.

Investigating the cause of this "warning rise" in the carrier rate, Glover (1920) was led to suspect a relationship between it and overcrowding in the sleeping huts. These huts were at the best poorly ventilated (Eagleton 1919-20), and during the stress of war the mobilization standard had been overstepped, so that beds, instead of being separated by 1 ft. 4 in., were practically touching each other. Glover (1920) noticed that the carriers in a given hut tended to be aggregated together; three Type II carriers were in adjacent beds, two Type I carriers, and so on. This pointed strongly to the direct transmission of the meningococcus from one man to another sleeping in the next bed. Finding that the spraying capacity during normal sleep was not more than about 3 ft., Glover tried the effect of spacing out the beds in the hope that the infection would be diminished. The results obtained were in accordance with expectation. The effect of the distance between the beds was not confined to the carrier rate. At Caterham depot, where there was severe overcrowding, an outbreak of cerebrospinal fever had occurred during each winter of the war, but subsequently to the adoption of the spacing-out policy in 1917-18, not a single case occurred.

Because of the far-reaching conclusions on the relation between overcrowding and the carrier rate, and the importance of detecting the "warning rise," Glover's work has received a great deal of attention.

In the Detroit epidemic of 1928-29, Norton and Baisley (1931) found no association between the degree of overcrowding in the home and the contact carrier rate. During the outbreak of 1931 at Aldershot, Armstrong and his colleagues (1931), from a study of the position of carriers in dormitory barrack rooms, were unable to obtain any evidence that infection occurred mainly at night. The carriers were scattered quite irregularly without any particular relation to the position of the beds. Our own nasopharyngeal surveys of the civilian population carried out before the second world war showed us that the carrier rate in institutions may be as high as 20 per cent. and over, without any outbreak of cerebrospinal fever occurring. Rake (1934) made the same observation. Perhaps the most striking figures, however, are afforded by Dudley and Brennan (1934). Working at the Chatham naval hospital, they found that between January, 1932, and March, 1933, there were 11 cases of cerebrospinal meningitis with a carrier rate of about 13 per cent. During the period March, 1933, to May, 1934, the carrier rate was 54 per cent., yet not a single case of meningitis occurred. During the same period at the Royal Naval Hospital, Portsmouth, there were 6 cases of meningitis with a carrier rate of only 5 per cent. Analysis of the distribution of carriers at Chatham showed no constant relationship between the density of the population and the carrier rate. The senior ratings with the most spacious sleeping accommodation had as high a carrier rate—60 per cent.—as the recruits with the worst sleeping quarters. Phair, Schoenbach and Root (1944) carried out a survey at an army camp in which cerebrospinal fever was prevalent. There were no cases, however, in the unit they examined. A group of 99 men was swabbed 28 times in 68 days; no fewer than 91 of these men were found on one or more occasions to be harbouring meningococci in the nasopharynx. The average carrier rate was 41 per cent.

occur in perhaps 30 per cent of all cases. Gonococcal meningitis occurs, perhaps more frequently than formerly thought.¹⁵

Once established, gonococcal infection persists for a long time; five to fifteen years' duration has been reported, but exclusion of reinfection is a difficult matter. Carpenter and Westphal,¹⁶ however, have observed infection of seven years' duration without reinfection. Following symptomatic cure by chemotherapy, gonococci may persist in the urethral secretions; Koch, Mathis and Geiger¹⁷ found, for instance, that nearly one-third of a group of 926 patients followed after apparent cure continued to carry gonococci. In general, very little is as yet known of the gonococcus carrier or the part played by the carrier in the spread of the disease.

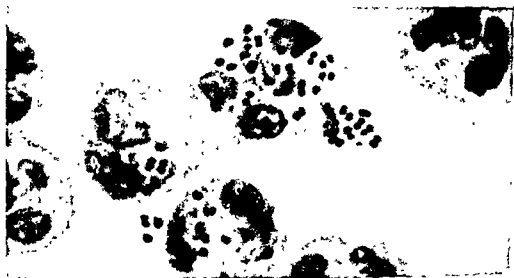


Fig. 58. Urethral smear from gonorrhea. Gram stain. Note the intra- and extra-cellular position of the gonococci and their typical coffee-bean shape and arrangement in pairs. $\times 2400$.

Gonorrheal vulvovaginitis occurs in epidemic form in little girls, and in these instances the infection is transmitted by bedclothes, towels, common bathtubs and other fomites. The commonest complications are urethritis, proctitis and cervicitis. Such epidemics are frequently exceedingly difficult to control and constitute a serious problem in many institutions such as children's wards in hospitals.¹⁸

Gonorrheal ophthalmia of the newborn is a well-known consequence of maternal infection, infection does not occur *in utero* but during passage through the birth canal. Although exact information is not obtainable, it is estimated that 10 per cent of all cases of blindness are traceable to this source, and that in the United States there are perhaps 12,000 children blind from this cause. The instillation of silver nitrate or other silver salts immediately after birth prevents infection.

¹⁵ Branham, Mitchell and Brainin. Jour. Amer. Med. Assn., 1938, 110 1804.

¹⁶ Carpenter and Westphal Amer. Jour. Pub. Health, 1940, 30 537.

¹⁷ Koch, Mathis and Geiger Ven. Dis. Inf., 1944, 25 35.

¹⁸ A comprehensive discussion of vulvovaginitis in children may be found in the Medical Officer, 1938, 59 191, 203, see also Cohn, Steer and Adler. Ven. Dis. Inform., 1940, 21.208.

perineural spaces of the olfactory nerves. Further work showed that material might pass from the nasal to the intracranial cavity by way of the blood vessels piercing the cribriform plate. Fine canaliculi, representing prolongations of the subarachnoid space into the olfactory mucosa, have also been described traversing the cribriform plate, homologous with similar prolongations found in the internal ear and around the optic nerve; but Clark was unable to satisfy himself that these canaliculi had any direct communication with the nasal lymphatic vessels. Methylene blue injected into the subarachnoid space beneath the frontal lobe, did not appear in the lymphatic vessels of the nose, but also the lymphatics of the nose are centrifugal, whereas those of the brain (not lymph) in the perineural spaces may, as Clark's observations have shown, be centripetal, and thus carry particulate material from the nose to the brain (see also Findlay and Clarke 1935, Rake 1937).

Another possibility is that the organisms may invade the nasal sinuses, set up a purulent sinusitis, and reach the skull by the lymphatics, or by direct extension through the bone. Numerous observers have found a sinusitis in cases of cerebrospinal fever, particularly of the sphenoidal sinus. Embleton and Peters (1915) examined three patients who had died of the disease, and found in each a sphenoidal empyema due to the meningococcus. Against this view are the facts that, though not uncommon, sinusitis is by no means constant in cerebrospinal fever, and that in children, in whom the disease is commoner than in any other class, the sphenoidal sinus does not develop before the third year of life. Moreover it is possible that the involvement of the nasal sinuses may be secondary to the meningitis, the organism being carried by the natural flow of the lymph stream to the nose. Flexner (1907b) found in monkeys, injected intraspinally, that the organisms might find their way from the meninges to the nasopharynx.

As well as the transthemoidal and the sphenoidal routes, it has been suggested that spread may occur via the Eustachian tube to the middle ear and thence to the brain. This seems unlikely because of the rarity of otitis media in cerebrospinal fever, and of the lateness of its development when it does occur.

The advocates of direct lymphatic spread from the nose to the meninges object to the hamatogenous route of infection on the ground that blood cultures are frequently sterile, that positive results are not more common at the beginning of the disease than during its later stages, and that the arthropathies and visceral lesions which sometimes appear as complications occur fairly late in the course of the disease, suggesting that the blood has become secondarily infected from the meninges; that this may occur in animals has been shown by Flexner (1907b), who found meningococci in the blood subsequent to intraspinal injection.

(b) THE HÆMATOGENOUS ROUTE.—Meningococci were found in the blood by Salomon in 1902; Jacobitz in 1905 reported two cases; Elser (1905-06) obtained 10 positive blood cultures out of 41—mostly in the first week of the disease; Dreu Bonné (1906) was successful in 4 cases out of 5. During the war of 1914-18 Baeslack (1918) and his co-workers cultivated the meningococcus 8 times from 22 cases, and Barber and Fleming (see Herrick 1918) 12 times from 15 consecutive cases. Moreover the organism has been cultivated from the petechial spots (Muir 1919), from the swollen periarticular lesions (Still 1898), from the endocardial vegetations, and from other situations clearly infected by the blood stream. Cases of chronic meningococcal septicæmia lasting for months, sometimes with ultimate recovery, are not uncommon (for references see Campbell 1943).

From these records it is clear that an infection of the blood is frequently present. It must be remembered that the meningococcus is not an easy organism to grow, and that if it is present in only small numbers the chances of obtaining it in blood culture are not great. With ordinary care, however, at least 25 per cent. of the blood cultures may be expected to be positive in the first week of the disease.

As well as the finding of meningococci in the blood, other reasons have been advanced in favour of the hamatogenous route. Clinical evidence shows that a meningococcal septicæmia may occur without the development of meningitis. Herrick (1918) states

Except in the case of vulvovaginitis in children, gonorrhea is spread by direct contact, usually sexual. Once infected, an individual may remain infective for a long time, and gonococci may persist in the genito-urinary secretions for years after apparently complete recovery, and even though they may not be found by bacteriological examination the infection may be transmitted. Gonorrhea persists, then, in the human population in a smoldering endemic form and, as a consequence of the nature of its transmission, widespread epidemics in the usual sense do not occur.

Bacteriological Diagnosis of *Gonococcus* Infection.¹⁹ The demonstration and identification of the gonococcus are essential to the diagnosis of gonorrhea and it should be borne in mind that every case of gonorrhea is potentially a medico-legal case. The presence of gram negative intracellular diplo-



Fig. 59 The oxidase test for the identification of gonococcus colonies. Pure culture on blood agar. Left, gonococcus colonies before the application of tetramethyl-*p* phenyl enediamine solution. Right, the same colonies after the application of the reagent. Note the greater intensity of color about the edges of the colonies immediately after application, and the discoloration of the medium. $\times 5$.

of the gonococcus gives a higher proportion of positive results than direct smear examination alone and cultured gonococci may, of course, be identified. The question of survival of this relatively delicate bacterium in transport of specimens is of some importance. No really satisfactory method of preserving specimens has been developed. Of the more recent work, it may be noted that Peizer and Steffen²⁰ found that immediate streaking of the swab on plasma hemo-

¹⁹ For a recent and detailed summary of the laboratory procedures see Carpenter, Ven. Dis. Inform., 1943, 24:133, also the summary of a panel discussion in Amer. Jour. Pub. Health, 1947, 37:1461.

²⁰ Peizer and Steffen: Ven. Dis. Inf., 1947, 28:218.

supernatant fluid are layered gently on to a similar quantity of monovalent or polyvalent antimeningococcal precipitating serum in a Dreyer's tube. Usually a fine white opaque disc of precipitation becomes apparent in a few minutes. If it does not, the tube is incubated in a water-bath for 1-2 hours at 37° C. Both Rake (1933b) and Macgrath (1935) obtained very satisfactory results with this method (see also Alexander and Rake 1937). In Macgrath's series of 120 cases, a positive reaction occurred in 116, whereas of 180 control fluids, all but 2 were negative. No other method affords such a rapid diagnosis.

(2) *Immediate plating.* As soon as the spinal fluid is withdrawn, it should be streaked in quantities of 1 ml. over 2 or 3 plates of blood agar, ascitic agar or Gordon's tryptagar (Gordon *et al.* 1916), or inoculated on to Loeffler's serum (Fairbrother 1947). The cultures should be incubated immediately, or if this is impossible, they must be transmitted to the laboratory in a warm container. The less their temperature falls below 37° C., the more likely is growth to occur, and the shorter is its lag period. Growth is often greatly improved by the addition of 5-10 per cent. CO₂ to the atmosphere. Colonies should be apparent within 18 hours. Their identity should be determined by microscopical examination for the presence of typical Gram-negative diplococci and by agglutination with specific antisera. If there is a heavy growth on the plates, this may be suspended in a small quantity of saline, heated to 65° C. for 1 hour, standardized to 500 million per ml, and tested against the type sera; incubation should be performed by the partial immersion method in a water-bath for 4 hours at 50° C. A more rapid result is said to be obtained by the use of specific antisera prepared by the injection of chickens; readings can be made in about 5 minutes (Phair, Smith and Root 1943). If the growth is insufficient for a tube test, or if an immediate answer is required, slide agglutination may be used instead (Bell 1920b), but the results should always be checked by the tube method. If there is insufficient growth on the primary plates to allow of either method of agglutination, subcultures must be made on to fresh plates, and the growth examined after 24 hours. The fact must not be forgotten that one variety of organism found in meningitis, *N. flavescens*, may produce a golden-yellow pigment (Branham 1930).

It must be pointed out that not all strains are readily agglutinable by the available type sera. Strains from sporadic cases are usually less readily agglutinated than those isolated during an epidemic. Much depends upon the strains selected for the preparation of the agglutinating sera (see Griffith 1917, Scott 1917, Bell 1920a, Murray 1929, Branham 1932, Rake 1933a).

It is advisable to test the fermentation reactions of the organisms isolated. For this purpose colonies should be inoculated into glucose, maltose, and sucrose peptone water containing 10 per cent. of ascitic fluid or blood serum. The meningococcus produces acid in glucose and maltose, but not in sucrose; *N. flavescens* is without action on any of the sugars. The final identification of any strain must be made on the basis of morphological, cultural, fermentation, and serological reactions.

(3) *Incubation of the spinal fluid with subsequent subcultivation.* It is always wise to incubate 5 ml. of the fluid at 37° C in a sterile tube, in case direct plating proves negative. Sometimes the organisms develop in the fluid itself, when they fail to do so in cultures made at the time of lumbar puncture. As soon as turbidity appears, the fluid should be subcultured on to suitable media, and the resulting colonies identified in the usual way.

globin agar in a screw cap bottle resulted in a high proportion of positive cultures, and these workers are of the opinion that the inclusion of dyes such as Nile blue or crystal violet in the medium may destroy gonococci as well as contaminants. For culture an infusion chocolate agar, developed by McLeod, or minor modifications of it such as the plasma hemoglobin modification studied by Thayer, Schubert and Bucca,²¹ is a medium of choice, and is inoculated directly with the specimen (or sediment if it is urine or spinal fluid). The culture must be incubated in 10 per cent CO₂; this atmosphere may be satisfactorily approximated by putting the plates in a jar together with a lighted candle and sealing or by the inclusion of a handful of moistened fresh oats in a sealed container with the cultures. The oxidase test serves to differentiate the oxidase-positive colonies and, if picked immediately, they may be subcultured. Identification is based upon sugar fermentations in serum broth.

Pathogenicity for Lower Animals. The gonococcus is non-pathogenic for lower animals, aside from the toxicity of the cell substance as noted above, and gonorrhea has never been reproduced in experimental animals, including anthropoid apes. An experimental infection of the anterior chamber of the rabbit's eye has been described by Miller and his co-workers²² in which the gonococci multiply and invade intraocular tissues, especially the ciliary body and lens, giving rise to a chronic infection in approximately one-third of the animals. This infection has been made use of in the study of the efficacy of chemotherapeutic agents, etc.

Immunity. Little if any immunity to the gonococcus is acquired as a result of infection, and second and third infections may be superimposed upon the first, i.e., acute upon old chronic infections. As might be expected, then, the therapeutic use of vaccines and various types of antisera is without effect. The significance of the observed extensive phagocytosis of gonococci by polymorphonuclear leucocytes is uncertain.

Some degree of immunological response is evident, however. Complement-fixing antibodies are usually present, and patients may give a marked skin reaction to suspensions of killed gonococci. A number of attempts have been made to utilize these responses in the immunological diagnosis of gonorrhea.²³ The complement-fixation test has shown some promise but is not generally used. The skin reaction is apparently too variable to have practical value.²⁴ It has also been observed that the discharges from gonorrheal inflammation give a precipitin reaction with antigenococcus serum, but this flocculation reaction has as yet no diagnostic value.

THE MENINGOCOCCUS²⁵

Inflammation of the meninges or investing membranes (pia-arachnoid) of the brain and spinal cord may be provoked by a variety of microorganisms, and may occur either as a primary affection or secondarily in the train of an infection originally begun elsewhere. One form of meningitis, characterized

²¹ Thayer, Schubert and Bucca: *Ven. Dis. Inf.*, 1947, 28:37.

²² Miller et al.: *Jour. Inf. Dis.*, 1945, 77:193, 201, 216.

²³ Cf. Casper: *Ven. Dis. Inform.*, 1941, 22:119.

²⁴ Cf. Torrey: *Jour. Immunol.*, 1940, 38:413.

²⁵ For general reviews see Murray: *Med. Res. Council Spec. Rept.*, Ser. No. 124, 1929, Branham: *Bact. Rev.*, 1940, 4:59.

(2) *Demonstration of Antibodies.*—The blood may be examined for specific antibodies in special cases; but the diagnostic value of such tests is very doubtful, and they must never be regarded as a substitute for direct cultural examination.

It has been found that agglutinins are frequently present in the blood serum of patients suffering from cerebrospinal fever (Dieudonné 1906, MacGregor 1910). Von Lingelsheim (1908) examined the sera of 593 patients. On the 1st day of disease 24.1 per cent. gave a positive agglutination reaction; from the 6th to the 20th day, 52.7 per cent.; after the 21st day, only 26.7 per cent. of positive reactions were obtained. The titre varies considerably. Bettencourt and França (1904) found that it was usually 1/10 to 1/50; occasionally titres of 1/200 or even 1/1000 were met with. Von Lingelsheim (1905a) gives the following figures:

146 agglutinated at	1/10
86 " " "	1/25
30 " " "	1/50
8 " " "	1/100
1 " " "	1/200
149 failed to agglutinate at	1/10

Gates (1918) found agglutinins almost constantly in the blood of carriers of 4 to 16 weeks' standing; the titre was generally 1/16 to 1/32.

It is seen that agglutinins are not present in more than 50 per cent. of cases as a rule. MacGregor (1910) found that when severe toxæmia was present, there were generally no agglutinins; likewise in chronic and in abortive cases. They were present in greatest quantities in cases with an acute onset and fairly high fever. Other observers (Elser and Huntoon 1909) have found the agglutination reaction to be too irregular for diagnosis.

Houston and Rankin (1907), studying the opsonic content of the serum of patients suffering from cerebrospinal fever, found that it was raised in 25 per cent. of patients on the 2nd day of the disease, in 60 per cent. on the 5th day, and in 96.1 per cent. after the 6th day. They stated that the opsonin test was of great value in the diagnosis of suspected cases. MacGregor (1910) found opsonins present in greatest quantity during the 2nd and 3rd weeks of the disease; like agglutinins, they are said to be most abundant in cases with acute onset. Cruickshank (1941) states that circulating antibodies can often be demonstrated in the second week of the disease, and perhaps even earlier in cases of meningococcal septicæmia.

Examination of Petechial Skin Lesions.

The presence of meningococci in petechial spots was demonstrated by Muir (1919), using the cultural method. In a considerable proportion of cases the organisms can be found microscopically in smears. McLean and Caffey (1931) were successful by this means in 14 out of 18 cases in children, and Tompkins (1943) in the Army in 39 out of 48 cases. The petechial spot—macules are unsatisfactory—should be punctured with a Hagedorn needle, while the surrounding skin is pinched so as to prevent the access of capillary blood to the lesion. Small drops of blood should be smeared on to a slide and stained with Giemsa. Typical intracellular diplococci with the adjacent surfaces flattened can be detected in this way, and confirmed by a Gram stain. They can often be found when blood culture is negative.

Post-mortem Examination.—In the examination of bodies at autopsy, it is important to take cultures within 12 hours of death if possible; the meningococcus is usually in smears from the puses, it is best to use the pus on the meninges; if there is no pus ... situation, the interior of the lateral ventricles should be carefully examined; occasionally some may be

especially by epidemic spread and usually designated as *epidemic cerebrospinal meningitis*, spotted fever or cerebrospinal fever, is caused by a specific micro-organism commonly known as the meningococcus.

This bacterium was described by Marchiafava and Celli in the meningeal exudate as early as 1884, but the first important work upon it was that of Weichselbaum, who, in 1887, obtained it in pure culture and described it in detail as the characteristic micrococcus found in six cases of acute cerebrospinal meningitis. Confirmation was supplied by the work of Jager in spite of some faulty observation. The etiologic role of the meningococcus has since been securely established by a number of investigations.²⁶

The meningococcus has been designated by a variety of names, including *Micrococcus meningitidis*, *Micrococcus intracellularis meningitidis*, *Neisseria meningitidis* and, according to Bergey (1948) *Neisseria intracellularis*. Al

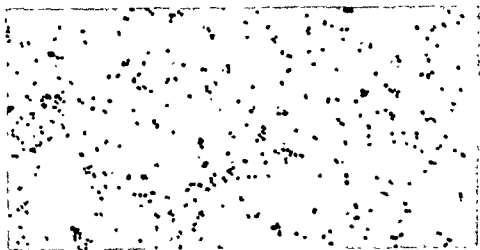


Fig. 60. Meningococcus, pure culture. Note the typical diplococcus arrangement. Fuchsin, $\times 1050$.

though *Neisseria* is generally accepted, common usage is divided between *intracellularis* and *meningitidis*.

Morphology and Staining. In film preparations of the meningeal exudate the meningococcus is very like the gonococcus and occurs in pairs or tetrads both within the leucocytes and free. The diplococci are flattened toward one another like gonococci, and there is considerable variability in the size of different cells in the same smear. In cultures the meningococcus averages a little less than $1\ \mu$ in diameter, and appears, as a rule, in pairs, short chains are seen more rarely. The variability in size observed in meningeal exudate may also be seen in cultures, particularly in those more than twenty-four hours old. Involution forms are common, and it is not unlikely that the larger cells are degenerative. Capsules are usually not apparent but become swollen in the presence of specific immune serum—the *Quellung* reaction. The meningococcus is non-motile and does not form spores.

Meningococcus colonies in blood agar are moist, elevated, smooth and with
²⁶ Cf. Foster and Gaskell *Cerebrospinal Fever*. Cambridge University Press, London, 1916.

vessels contaminated with nasal mucus. It is difficult to say how frequent such indirect infection may be, but in view of the extreme susceptibility of the meningococcus to drying and to cold, we may regard it as negligible. Neither in barracks nor in civilian households is this method of infection to be compared with the direct infection by nasal mucus sprayed from the carrier.

In reference to the prevention of cerebrospinal fever much has been written on the advisability of detecting and isolating carriers. Hachtel and Hayward (1911) were able to eliminate the disease from an orphan children's home by isolating the carriers, treating them with topical applications of anti-meningococcal serum, and not releasing them till after two negative swabs. Apart from occasional instances of this sort it will be realized that, however effective such an isolation policy might be, its rigid application in civil or military life would be quite impracticable, owing to the high proportion of carriers in any given population. Wherever possible, however, it is advisable to prevent carriers from coming into contact with infants and young children, particularly in dormitories and bedrooms.

In preventing an outbreak, or in limiting its spread, it is desirable on general grounds to reduce overcrowding as much as possible, to ensure adequate ventilation, and to induce people, as far as the weather and other conditions permit, to lead an open-air life and to avoid undue fatigue. Flack (see Report 1917) found that carriers became negative much more rapidly during fine weather and sunshine than during dull rainy weather.

Chemoprophylaxis.—Attempts at nasal disinfection of carriers with a view to preventing further spread of the disease were made during the war of 1914-18 without any conspicuous success, though light carriers were sometimes cured by intensive inhalation of a 1 per cent. zinc sulphate or 0.1 per cent. acriflavine spray. Far more hopeful are the results recorded by Fairbrother (1940) from the use of sulphapyridine. Convalescents who had received the drug during the acute stage of their illness were found, on nasopharyngeal swabbing, to be free from infection; and healthy carriers who were given a short course of treatment cleared up rapidly. It is possible that sulphapyridine or sulphathiazole in the form of nasal snuff might achieve the same end. During the second world war chemoprophylaxis was tried on a large scale in service units.

Kuhns, Nelson, Feldman and Kuhn (1943), for example, tried this method during an outbreak of cerebrospinal fever at two military depots in the United States. At Camp A 1 gm. of sulphadiazine was given three times a day to a group of 8,000 men; 9,300 men served as a control camp group. The carrier rate in the treated group fell immediately from 36 per cent. to 3.1 per cent., and never rose during the next 8 weeks above 7.2 per cent. In the control group the carrier rate, which was originally 38 per cent., never fell below 30 per cent. and at the end of 8 weeks was 55.8 per cent. as against 5.4 per cent. in the treated group. During the same period no cases of cerebrospinal fever

twice a day for 3 days; 9,500 men served as a control group. The carrier rate in the treated group fell at once from 30 per cent. to 0 per cent., and never rose during the next 8 weeks above 2.1 per cent. In the control group, the carrier rate, which was originally 29 per cent., never fell below this figure, and at the end of 8 weeks was 33.3 per cent. as against 0 per cent. in the treated group. Two cases of cerebrospinal fever occurred in the treated group, and 17 in the control group. It is to be noted that in each instance the treated and control groups were kept separate, that all persons were treated simultaneously, that new admissions were given sulphadiazine, and that precautions were taken against the

a bluish gray tinge. They do not produce green discoloration or hemolysis and may be readily differentiated from the hemolytic and viridans streptococci and the pneumococcus. The colonies are not so white and opaque as those of the staphylococci.

The meningococcus stains readily with the usual aniline dyes and, like the gonococcus, is gram-negative. The involution forms found in cultures tend to stain unevenly, of course, but even young cells may show the presence of metachromatic granules when stained by Löffler's alkaline methylene blue and other stains, and to a greater extent than the gonococcus. It may be noted that no sure distinction between the meningococcus and the gonococcus can be made on morphological grounds, and the identification of gonococci in gonococcal meningitis is dependent upon culture and differential fermentations

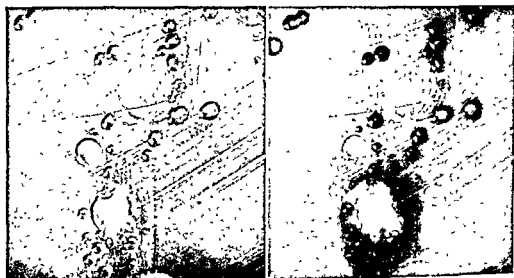


Fig. 61. The oxidase test for the identification of meningococcus colonies. Mixed culture on blood agar. Left, colonies of meningococci and contaminants before the application of tetramethyl *p*-phenylenediamine solution. Right, the same colonies after the application of the reagent. Note that the meningococcus colonies show the development of color first about the edges and there is slight discoloration of the medium. $\times 5$.

Physiology. Strains of meningococci vary considerably in the ease with which they may be cultivated; some strains will grow, although sparsely, on nutrient and infusion media, but in general rich media containing serum or whole blood are required. Infusion base chocolate agar or blood agar are the most useful media. The hydrolyzed casein-starch medium of Mueller and Hinton and Boor's tryptic digest medium will support growth of this bacterium. According to Boor the meningococcus differs from the gonococcus in that it does not require the addition of cystine to his medium. Frantz²⁷ has found that stock cultures of the meningococcus, which are, however, less exacting than recently isolated strains, will grow on an inorganic salt-glucose medium containing glutamic acid and cystine, and others have reported that such strains require only glutamic acid and glucose or lactate.²⁸ In isolating the meningococcus it is essential that the culture medium be warm when inoculated and kept warm until finally placed in the incubator. Growth is favored, espe-

²⁷ Frantz: Jour. Bact., 1942, 43 757.

²⁸ Grossowicz Jour. Bact., 1945, 50 109.

of all ages with a case-fatality rate of 8.6 per cent. ; this figure included every case admitted, even those dying within a few minutes of reaching hospital. Sulphapyridine was used for 471 of the cases, sulphathiazole for the remaining 29. Very similar figures were quoted by Banks (1948) using mainly sulphathiazole and sulphadiazine. The fatality rate is much higher in infants and in those over 60 years of age than at other times of life when it is reduced to between 3 and 6 per cent. (see Harries 1942, Beeson and Westerman 1943, Report 1944, Banks 1948). There is no satisfactory evidence to show that a combination of sulphonamides and serum offers any advantage over sulphonamides alone.

Pneumococcal Meningitis

This form of meningitis occurs as a complication of lobar or broncho-pneumonia, and is commoner in children than in adults. Not infrequently in children it is secondary to middle-ear disease. Sometimes a primary pneumococcal meningitis is seen. The pneumococci are present in large numbers in the cerebrospinal fluid, which is turbid and full of pus cells ; the cocci may be recognized by their appearance as Gram-positive, lanceolate, capsulated, and mostly extra-cellular diplococci. Cultures are best taken on to blood agar or ascitic agar. The type of coccus may be determined in the usual way. In untreated cases the disease is almost uniformly fatal. Serum treatment with univalent type serum may be tried, preferably by the combined intravenous and intrathecal routes, but far more favourable results are to be expected from the administration of the sulphonamides (see Coleman 1940) and of penicillin.

Streptococcal Meningitis

Streptococcal meningitis is most frequently due to extension from middle-ear disease, especially in children, and to perforated wounds of the skull. The cerebrospinal fluid is turbid, and contains large numbers of pus cells and of streptococci, which are usually of the β -haemolytic type. Before the sulphonamide era recovery was regarded as exceptional, though Watson-Williams (1938) points out that early mastoidectomy, combined with repeated lumbar puncture and the administration of antistreptococcal serum, may result in a substantial proportion of recoveries. In practice, sulphonamide or penicillin therapy should be instituted as early as possible, along with appropriate surgical measures.

Tuberculous Meningitis

Tuberculous meningitis is probably always secondary to a tuberculous focus in some other part of the body, but, as it is often difficult to determine the site of the primary lesion, even at autopsy, the disease not infrequently appears to be primary. Osler (Osler and McCrae 1920) states that it may occur during the 1st year of life, but is commonest between the 2nd and 5th years ; it is, however, not uncommon in adults, particularly as a terminal infection in pulmonary tuberculosis. The laboratory diagnosis is often difficult. Lumbar puncture reveals a fluid which may be limpid, and may contain only mononuclear cells. The protein content is raised, the sugar, and usually the chloride, content are lowered. The cell count generally ranges between 50 and 400 per c.mm. Lymphocytes predominate but are accompanied by polymorphonuclear and often some plasma cells. If the fluid is allowed to stand at room temperature, a clot not infrequently forms ; this should be spread on a slide, and stained by Ziehl-Neelsen. If no clot

cially in primary isolation, by incubation in an atmosphere containing 10 per cent carbon dioxide. The meningococcus will grow over a temperature range of 25° to 42° C. with an optimum at 37° C. Although sparse growth will occur under anaerobic conditions, the meningococcus is, for all practical purposes, an aerobe.

Continued cultivation on laboratory media results in more luxuriant growth, and the bacteria presumably become less nutritionally fastidious. Meningococcus cultures are difficult to keep, however, and tend to die out in stock culture. In most media these bacteria die within a few days if not transferred, but vitality may be preserved for several weeks in stab cultures in starch agar (1 per cent corn starch in nutrient agar) and are best kept in the incubator.

The relatively early appearance of involution forms in meningococcus cultures as well as their limited viability when not transplanted is, perhaps, attributable to their formation of an active autolysin, and in saline suspension in the incubator autolysis may take place within a few hours. The autolysin is heat-labile, being destroyed at 65° C. in thirty minutes, and suspensions prepared for agglutination studies should be inactivated in this way.

The meningococcus, like the gonococcus, is a delicate microorganism and not highly resistant to deleterious influences. It is killed in a short time by drying and by exposure to dilute disinfectants. It is particularly sensitive to heat and cold and, unlike many bacteria, dies out within a few days at 0° C.

The meningococcus is not an active fermenter. Considerable quantities of acid, presumably lactic for the most part, are formed from glucose and maltose. The fermentation of maltose serves to distinguish the meningococcus from the gonococcus. Neither is this bacterium actively proteolytic, for coagulated serum is not liquefied.

Toxins. Meningococcus meningitis in man and that reproduced in experimental animals is usually accompanied by a profound toxemia. The meningococcus, however, appears to form no soluble toxin, though its cell substance is toxic to experimental animals when injected in relatively large amounts. Miller and his co-workers²² have shown that the toxicity is heat stable (100° C. for thirty minutes) and the rate of its destruction suggests that the endotoxin consists of two substances, one much more thermostable than the other. The relationship of the endotoxin to the "P" substance (see below) is not clear.

Variation. Rough and smooth colony types of the meningococcus have been described by Bake,²³ who found that recently isolated strains were generally smooth while old stock cultures were rough. Mucoid colonies were observed in a few instances. The change from smooth to rough was associated with a partial loss of immunological type specificity.

Classification. The meningococci are closely related to the gonococci not only morphologically and physiologically but immunologically, in that certain antigenic substances appear to be held in common as indicated previously.

The meningococci are not themselves immunologically homogeneous, as was found by Dopter²⁴ that certain strains culturally "typical" were

²² Miller et al. Jour. Inf. Dis., 1943.

²³ Bake Jour. Exp. Med., 1933, 57.

²⁴ Dopter. Cerep. rend. Soc. bul.

by culture, and by demonstration of the specific polysaccharide in the spinal fluid either by a precipitin or a hæmagglutination test (Warburton, Keogh and Williams 1949). Treatment with streptomycin, penicillin, and sulphonamides greatly lowers the case-fatality rate. Even better results, it is claimed, may be obtained with chloramphenicol. Prather and Smith (1950) treated 15 consecutive cases with this drug without a single death.

Non-suppurative Meningitis

We may refer here to a form of meningitis that follows contamination of the cerebrospinal fluid during lumbar puncture and spinal anaesthesia. It is usually of a low-grade type—sometimes referred to as serous meningitis—but fatal cases occur from time to time. The disease is caused by organisms which under ordinary conditions are non-pathogenic, such as *Pseudomonas* and *Achromobacter*. Contamination is often due to the use of so-called sterile water for rinsing syringes. To prevent this totally unnecessary disease, strict asepsis should be practised. All apparatus used for lumbar puncture should be sterilized by heat—preferably dry heat—and water for rinsing and cooling purposes, except from sealed autoclaved bottles used on one occasion only, should be avoided (see Smith and Smith 1941, Garrod 1946, Report 1948).

Other Forms of Meningitis

Occasionally meningitis may be caused by organisms, such as *B. anthracis*, *Bact. coli*, Friedländer's bacillus (Gordon and Norton 1930), *Salm. typhi*, *Salm. enteritidis* (Stevenson and Wills 1933), *Salm. paratyphi B* (Patterson 1942), *Pf. mallei*, *Br. suis* (Hartley *et al.* 1934, Hansmann and Schencken 1932), *Br. abortus* (Magoffin *et al.* 1949), the gonococcus (Strumia and Kohlhas 1933, Branham *et al.* 1938), *Diplococcus mucosus* (Cowan 1938, Bray and Cruickshank 1943, Christie and Cook 1947), *Actinomyces bovis* (Henry 1910), *Leptospira icterohæmorrhagæ* (Marie and Gabriel 1935, Mollaret and Erber 1935) and *Leptospira canicola* (see Chapter 82).

For meningitis due to filtrable viruses, see Rivers and Scott (1935) and pp. 2162-5.

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cocci, did not agglutinate with antimeningococcus serum. These he designated "parameningococcus," a term that has since given rise to some confusion and may well be discarded. Gordon and Murray³² distinguished four serological types, which they designated by Roman numerals. These types have been telescoped into two groups, Group I containing Types I and III which are very closely related, and Group II made up of Types II and IV. Type IV seems to have disappeared (it is doubtful whether the European Type IV and American Type IV were identical) in recent years and Group I and Type II are synonymous. There is considerable variation in the frequency of occurrence of the two groups, and in general Group I appears to be associated with the epidemic disease while Group II predominates in interepidemic years. Recently a new type, related to Type II but independent and homogeneous, has been found to be quite prevalent.³³ It has been designated Type IIa. The types of meningococci encountered at present are, then, Group I, Group (or Type) II, and Type IIa.

While it is not uncommon to encounter strains of meningococci which do not fall into these types, most strains may be assigned to one or another of them. In practice typing is complicated by the merging of types and the antigenic instability of meningococcus cultures. For routine identification agglutination or capsular swelling (*Quellung*) with polyvalent antiserum suffices.

Studies on the nature of the antigens of the meningococcus by Rake and Scherp³⁴ showed that three types of antigen are present. One is a polysaccharide common to all types of meningococci and found in the gonococcus also, which was designated "C" substance. A second fraction, likewise common to all meningococcus types and highly toxic to rabbits, is protein in nature and designated "P" substance. A third, which is found in Types I and III is polysaccharide in nature. A substance from Type II appears to be a protein.

Kabat, Kaiser and Sikorski³⁵ have prepared the polysaccharide from Type I.

While the routine typing of meningococci is probably not worth while, typing in connection with the preparation of therapeutic antisera is obviously of importance, and at the present time such antisera are generally polyvalent.

Pathogenicity for Man. The resistance of man to meningococcus infection is relatively high, and the incidence of healthy carriers is invariably considerably higher than that of cases of the disease. It is probable that predisposing factors play a large part in determining whether or not infection will occur; insufficient clothing, inadequate ventilation, exposure to inclement weather and fatigue very likely contribute in large measure to increasing susceptibility. In 1945, 7305 cases and 1539 deaths from meningococcus meningitis were reported by 44 states, rates of 6.2 and 1.3 per 100,000 population respectively; this is a decline from the record peak incidence in 1943 of 14.1 per 100,000.

The meningococcus is initially present in the nasopharynx and from there

³² Gordon and Murray: Jour. Roy. Army Med. Corps, 1915, 25 411.

³³ Branham and Carlin: Proc. Soc. Exp. Biol. Med., 1942, 49 141.

³⁴ Rake and Scherp: Jour. Exp. Med., 1933, 58:341, 361.

³⁵ Cf. Menzel and Rake: Jour. Exp. Med., 1942, 75:437.

³⁶ Kabat, Kaiser and Sikorski. Jour. Exp. Med., 1944, 80 299.

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gains access to the central nervous system. The route by which this occurs is uncertain, it is thought by some that the bacteria follow the perineural spaces of the olfactory nerves or set up a preliminary sinusitis and reach the brain either via the lymphatics or by direct extension through the bone. Others believe the meningococci reach the central nervous system via the blood stream through a preliminary bacteremia. While there is no definitive evidence concerning the means by which meningococci reach the central nervous system from the nasopharynx, the evidence appears to favor the hematogenous route. Occasionally the infection in the nasopharynx may extend into adjacent areas, giving rise to conjunctivitis, pneumonia, etc.

In the healthy carrier the infection remains confined to the nasopharynx and in this case is short lived there and produces few or no symptoms. When the blood stream is invaded early in the disease hemorrhages usually occur in

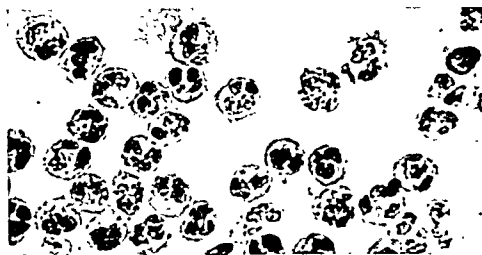


Fig. 62 Meningococci in spinal fluid, showing phagocytosis of the microorganisms. Gram stain, $\times 1050$

the skin and petechiae appear, especially on the wrists and ankles, or on any mucous or serous surfaces. These are apparent in twenty four hours after invasion and fade in a few days. The rash is quite different from other purpuric rashes, the spots take geometric shapes and are highly irregular in size. Meningococci may be observed in smears of material taken from these lesions. Other symptoms include sudden onset, chills, fever, and meningeal symptoms such as headache, drowsiness, etc. Pain in the arms and legs is common.²⁷ The invasion of the blood stream may take the form of a fulminating meningococcemia (the Waterhouse-Friderichsen syndrome) with adrenal apoplexy as the immediate cause of death and massive bilateral hemorrhage of the adrenals as the outstanding pathology. This form of meningococcal disease is uncommon—little more than 200 cases have been reported—and occurs much more frequently in infants than in adults. Its sudden and violent character with rapid fatal termination has led to deaths due to this cause being classified as suspicious. The bacteremia may also take a more chronic form, and give rise to a purulent synovitis or meningococcal arthritis.

²⁷ For a general discussion see Strong, *Amer. Jour. Med. Sci.*, 1943, 206:561.

Uffreduzzi 1894, Neisser 1894), in tenosynovitis (Hocheisen 1906), in vesiculitis (Wynn 1905), in cystitis, in ophthalmia (Neisser 1879), less frequently in peri-renal abscesses (Colombini 1898), in pyelonephritis, and in peritonitis.

In rare instances the disease may be very acute, the organisms gaining access to the blood stream and giving rise to a bacteræmia, which may be followed by pyæmia. In these cases, which may prove fatal, there is widespread infection throughout the body, and *post mortem* there may be found endocarditis, myocarditis, less frequently pericarditis, subcutaneous and intramuscular abscesses in different situations, polyarthritis, phlebitis, pleurisy, general adenitis, pneumonia, and septic infarcts in the spleen and kidneys. Several such cases have been reported (Leyden 1893, Ghon and Schlagenhauser 1898, Thayer and Lazear 1899, Strong 1904, Duval and Lewis 1905, Wynn 1905, Hocheisen 1906, Jenkins 1922). Gonococci have been demonstrated in nearly all these lesions, and have often been obtained in pure culture from the blood stream both during life and after death (Thayer and Lazear 1899, Duval and Lewis 1905, Wynn 1905, Jenkins 1922). Meningitis is a rare complication (see Strumia and Kohlhas 1933). A few cases are on record of a gonococcal stomatitis (Crosby 1905), or parotitis (Colombini 1898); from the latter situation the organisms have actually been cultivated.

Gonorrhœa in Children.—Children suffer from ophthalmia neonatorum and from vulvo-vaginitis. The former is due to infection at birth with gonococci present in the maternal passages, the latter to infection of towels in infants' hospitals: but not all cases of ophthalmia neonatorum and vulvo-vaginitis are of gonorrhœal origin, and not all Gram-negative cocci found in these diseases are gonococci.

At one time ophthalmia neonatorum was responsible for a large proportion of cases of blindness, but since the introduction of the Credé preventive treatment it has become much less common.

Vulvo-vaginitis is mainly an institutional disease. Like puerperal fever and the old hospital gangrene, it is very much more frequent in institutions where large numbers of patients are collected together. Thus Fischer (1895), reporting in 1895 on the incidence during the past 2 years of the disease in the children's hospital at Altona, said that of 50 cases all but 10 were contracted in the hospital; of these 10, some had apparently been imported from other hospitals. Holt (1905) reported on no fewer than 273 cases of the disease in the Babies' Hospital, New York, during the preceding 11 years. The disease is spread from one cot to another by the use of towels and other linen that are imperfectly sterilized. Once introduced into a hospital or institution, it is a most difficult disease to eradicate; only scrupulous care in technique, constantly maintained for months on end, will prove successful eventually. The disease has a way of cropping up after it has lain dormant for weeks; to relax precautions too soon is but to court disaster. By no means all cases of vulvo-vaginitis in children are of gonococcal origin. Other organisms, such as *Staphylococcus aureus*, are often responsible, and a correct diagnosis of gonorrhœal infection should therefore never be made unless the gonococcus is demonstrated. For a review of the disease, see Benson and Steer (1937).

It will be seen that the parts of the body attacked are not the same in adults as in children. Gonorrhœal ophthalmia is common in infants, rare in adults; gonorrhœal vaginitis is common in infants and children up to 5 or 6 years old, but is uncommon in adults. A similar difference is noted in the complications; thus in adults the internal genito-urinary organs are frequently attacked; in children

1904 and had not wholly ended in 1913. In 199 cases in which antiserum was administered between the first and the third day of the disease the mortality was 18 per cent. Variation in the case fatality rates in various epidemics is wide, however, and it is difficult if not impossible to determine the relative effect of differences in the virulence of the meningococci, variation in the efficacy of the sera employed and differences due to early or late administration of the serum.

The use of polyvalent antiserum is, of course, a necessity, as pointed out earlier. Continued experience with antimeningococcus serum tends to emphasize some of the difficulties of practice and interpretation. Antiserum is standardized by the mouse protection test, using meningococci in mucin suspension as the challenge inoculum. In spite of the beneficial results frequently



Fig. 65. *Neisseria catarrhalis*. Smear from a pure culture. Note the diplococci and elongated forms which have not yet divided. Fuchsin, $\times 1050$.

reported from the use of antiserum, there are some cases and some epidemics in which the serum treatment seems of little avail.⁵⁰

Prophylactic vaccination with suspensions of meningococci has been attempted by a number of workers, but the procedure is difficult to evaluate. For example, Maclean and Bevan⁵¹ found no evidence of the efficacy of active immunization in their study of an epidemic in Cyprus, while Genevray⁵² has reported highly encouraging results in Tonkin. In general there appears to be no consistent and unequivocal evidence of a significant degree of protection conferred by active immunization procedures.

OTHER GRAM-NEGATIVE DIPLOCOCCI

In addition to the gonococcus and the meningococcus, two other species of non-pigmented gram-negative diplococci are generally recognized.

Neisseria catarrhalis is found commonly in the nasopharynx of healthy individuals as well as of persons suffering from colds and other respiratory

⁵⁰ The treatment of meningococcus meningitis is discussed by Branham: Pub. Health Repts., 1938, 53:645.

⁵¹ Maclean and Bevan: Proc. Roy. Soc. Med., 1939, 32:1551

⁵² Genevray: Rev. méd. franc. d'extreme-Orient, 1941, 19:143.

a suitable medium (see Chapter 23). In *chronic* cases it may be extremely difficult to demonstrate the gonococcus in films, or to isolate it in culture. This holds particularly true for the female, where a diagnosis on microscopic grounds alone can rarely be made. Appropriate measures must be taken to obtain material for examination from the situation most likely to be the seat of chronic infection—the deep urethral glands and the prostate in the male, the Bartholinian glands and the cervical glands in the female. According to Koch (1948) cultures from the cervix of females suffering from acute gonorrhoea are more likely to be positive during the oestrogenic phase of the menstrual cycle when the cervical mucus is about neutral in reaction than during the non-oestrogenic phase when it is acid. It may be noted, that the fluid withdrawn from the joints in gonorrhoeal arthritis is frequently sterile, and that the same is true of pus withdrawn from the Fallopian tube in chronic cases of gonorrhoeal salpingitis.

For diagnostic cultivation the method described by McLeod and his colleagues (1934) is to be strongly recommended. The medium used is a 10 per cent. heated blood agar prepared from broth made according to Wright's (1929, 1933) method, *i.e.*, extraction of the minced meat for 45 minutes at 60° C. in the presence of 1 per cent. peptone and 0.2 per cent. Na_2HPO_4 , followed by steaming. The broth should have a reaction of pH 7.4, and the minimum amount of agar necessary to give stability to the medium should be added. The plates are first incubated for 18 hours at 36° C. in a closed jar containing air, 8 per cent. of which has been replaced by CO_2 , and then for 24 hours under ordinary aerobic conditions. A 1 per cent. solution of tetramethyl-p-phenylenediamine hydrochloride is then poured over the plate and run off immediately. The effect of this solution is to turn gonococcal colonies a bright purple colour. Medium-sized convex translucent colonies that rapidly turn bright purple and are found microscopically to consist of Gram-negative diplococci may be provisionally accepted as gonococci. For further identification, when necessary, the colonies are picked off, and studied culturally and biochemically. A rapid provisional method for establishing their identity based on the solubility of gonococci in N/10 NaOH is described by Cantor, Shelanski and Willard (1942). Occasionally colonies giving the oxidase reaction and consisting of Gram-negative diplococci are isolated from the genito-urinary tract which subsequent examination shows are not true gonococci. Organisms of this type are unlikely to give rise to more than 1-5 per cent. of erroneous diagnoses (see Wilkinson 1952). Cultural methods are greatly superior to direct microscopical examination of smears (see, for example, Gibbons 1937, Carpenter, Leahy and Wilson 1938, Sewell, Clarke and Nelson 1941, McLeod 1947); nevertheless a certain number of cases do occur in which the smear method is positive when the cultural method is negative. The greatest caution, however, must be taken in chronic cases in identifying the gonococcus on morphological appearances alone. As Beech (1933) points out, confusion is often caused by staphylococci and streptococci, which may occur in pairs and fail to retain the Gram stain.

If swabs for cultural purposes cannot be inoculated at once on to a suitable medium, measures must be taken to prevent death of the gonococci. Cox and McDermott (1943) recommend collection of the exudate on to a small wooden toothpick; this is dropped into a tube, 50 × 6 mm., containing 0.6 ml. of a mixture made up of equal parts of sterile distilled water and defibrinated horse blood to which 1/30,000 crystal violet and 5 mgm. per 100 ml. of *para*-aminobenzoic acid are added. The tube is closed with a corked paraffined stopper. By this means successful results may be obtained, even after transmission through the post. Some workers prefer 1/5,000 thallium acetate to crystal violet (Cooper *et al.* 1950). Moffett, Young and Stuart (1948) use a wooden swab dipped before sterilization into a 1 per cent. watery solution of finely ground charcoal. After the sample has been taken, the swab is transferred to a small screw-capped bottle containing a buffered

infections. The cells as a rule are somewhat smaller than those of the meningococcus. Growth occurs on ordinary nutrient agar much more readily than in the meningococcus, and the colonies are generally thicker and more opaque. Dextrose and other sugars are not fermented. Different strains vary in their pathogenicity for animals, but many strains are fully as pathogenic as meningococci for white mice. In man they appear at times to excite catarrhal inflammation and sometimes pneumonia and meningitis have been reported. They seem to have been conspicuous invaders in the 1918 influenza epidemic in some localities.

Neisseria sicca is a small gram-negative coccus found on the mucous membranes of the respiratory tract. It grows at room temperature as well as at 37°C.

in the blood stream of patients ill with clinical endocarditis.

The Pigmented Forms. Gram-negative diplococci which form a pale greenish-yellow pigment often best observed by transmitted light may be found in the upper respiratory tract of man. Formerly regarded as non-pathogenic, one species, *N. flavescens*, has been described as occurring in the spinal fluid in cases of clinical meningitis, as indicated earlier. These pigmented forms are differentiated from one another on the basis of fermentation.

The Obligate Anaerobic Species. Three species of *Neisseria* are obligate anaerobes, *N. discoides*, *N. reniformis* and *N. orbiculata*. These forms are parasites of man and are found in the mouth, intestinal tract and genital-urinary tract, but are not of known pathogenicity. Obligate anaerobes sometimes related to *Neisseria*, but differing in that the cells occur in pairs and sometimes in short chains rather than in pairs, are placed in the genus *Vibrio*. There are two species, *V. parvula* and *V. gazogenes*, formerly known as *Micrococcus parvula* and *Micrococcus gazogenes*. The former is weakly hemolytic and ferments glucose, while the latter is non-hemolytic and does not ferment carbohydrates. Both appear to be harmless parasites of man and are found primarily in the mouth and intestinal tract.

highest percentage of positive results. The test is therefore of considerable value in those cases in which microscopic and cultural tests are most likely to fail (see Tulloch 1929, Price 1933). It is specially useful in chronic pelvic disease.

Since the introduction of sulphonamide and penicillin treatment the value of the complement-fixation test has greatly diminished. The experience of numerous workers has shown that the proportion of positive results obtained now is far less than before, presumably owing to the failure of antibody formation in effectively treated cases. In some laboratories the test has been almost completely abandoned (Cooper *et al.* 1950).

Precipitin Reaction.—This method was advocated by Robinson and Meader (1920) who found that discharges from gonorrhœal inflammations gave a precipitin reaction with antigonococcus serum, even when no gonococci were to be found microscopically. Kelley (1922), who tried this reaction, was unable to confirm their results. The test cannot yet be relied on for diagnosis. A more specific sero-flocculation test was described by Saint-Prix (1950), who used a special extract of gonococci.

Meincke (1931) introduced his *Klärungsreaktion* (MKR test) and Muller (1932) his *Ballungs-Reaktion* (Im.B.R. test) for the diagnosis of gonorrhœa. According to Schröpl (1934), who made comparative observations on these tests, both of them give a higher proportion of non-specific reactions than the complement-fixation test, especially with syphilitic sera. One or other of them may be used, however, in conjunction with the complement-fixation reaction in order to amplify and control the results obtained by this test.

Skin Reaction.—Bruck (1909) found that patients with gonorrhœa showed a skin reaction to dead gonococci. Kohler employed this test in diagnosis, and found it to be valuable in many cases.

Irons (1912) prepared a glycerinated suspension from several strains of gonococci, which he called gonococcin. Inoculation was made on the skin with the point of a needle. After a few hours, in positive cases, a papule appears, surrounded by hyperæmia, reaching its maximum size in 24 hours, and disappearing after several days. The diameter of the papule and the hyperæmic zone around it is 5 mm. or more. The reaction was found to be generally positive at some time during the course of the disease, but in cases of severe infection, it might remain consistently negative. On the whole, it does not appear to be sufficiently regular to be of value in diagnostic work.

Prevention and Treatment.

Prevention is intimately associated with early diagnosis and efficient treatment. The mode of infection indicates sufficiently clearly the ways in which the disease can be avoided, and the reservoirs of infection which exist in any community. The problem is one of personal and social hygiene. The prophylactic use of oral penicillin administered within 2 hours of exposure to infection is said to cut down the incidence considerably (Eagle *et al.* 1919).

Various methods are recommended by different observers for preventing ophthalmia neonatorum of gonorrhœal origin. The Credé procedure, which consists in instilling drops of 1 per cent. silver nitrate solution into the eyes after birth, is still widely practised, though the additional or alternative use of penicillin and sulphonamides has taken its place in some clinics. At the Harlem Hospital in New York a combination of silver nitrate drops with sulphathiazole by the mouth or 50,000 units of aqueous penicillin intramuscularly proved very successful in practice (Watts and Gleich 1950).

In the treatment of gonorrhœa vaccines and antisera are no longer used (for references, see 3rd edition, p 1459).

THE ENTERIC BACILLI: THE COLIFORM BACTERIA, FRIEDLÄNDER'S BACILLUS, AND PROTEUS

The gram-negative, non-spore-forming bacilli make up a large group of bacteria which includes intestinal commensals such as the colon bacilli and *Proteus*, the enteric pathogens such as the typhoid, paratyphoid and dysentery bacilli, certain saprophytic forms and plant pathogens; and, as more distant relatives, the hemophilic bacteria (*Hemophilus*); the so-called hemorrhagic septicemia group (*Pasteurella*); and the causative microorganisms of undulant fever (*Brucella*).

By far the largest of these subdivisions is that of the enteric bacilli whose habitat is the intestinal tract of man. Some are, for the most part, intestinal commensals with but feeble pathogenic powers while others are highly pathogenic, producing intestinal disease of greater or lesser severity. The cholera vibrio is sometimes considered as somewhat set apart from the enteric group proper because of its curvature; this would appear to be an overemphasis of a minor morphological difference for not only do the longer enteric bacilli often show a slight curvature, but after continued cultivation on laboratory media the cholera vibrios lose their curvature and become morphologically indistinguishable from the other enteric bacilli. Still other bacteria are closely related to the enteric bacilli, in fact sometimes cannot be distinguished from them with certainty, but are set apart by differences in habitat. Such are Friedlander's bacillus, a common inhabitant of the upper respiratory tract and the causative agent of a small proportion of pneumonias, and the plant pathogens of the genus *Erwinia* which produce soft rots of vegetables. Similarly, the members of the genera *Proteus* and *Pseudomonas* occur as free-living saprophytes as well as intestinal commensals of occasional pathological significance.

The taxonomic relationships of these forms is shown in the accompanying scheme in which the pathogenic enteric bacilli are included under the general head of Enterobacteriaceae together with Friedlander's bacillus, but *Erwinia* and *Serratia*, the latter chromogenic saprophytes of which *Bacterium prodigiosum* (*Serratia marcescens*) is the best known representative, are omitted. It may be noted that the formal speciation adopted by Bergey (1948) does not correspond too well with the kinds of bacteria included under the head of Eschericheae.

The enteric bacilli fall into certain natural groups on the basis of physiological, immunological and pathogenic characteristics. These are: the coliform group which includes *Bacterium coli* and *Bacterium aerogenes* together with intermediate and variant forms; the *Salmonella* group made up of the

in association with this disease. The introduction of serological methods of differentiation seemed to offer a better hope of solving this problem, and Moser and von Pirquet (1902), Meyer (1902) and Rossiwall and Schick (1905) reported that scarlatinal strains of hæmolytic streptococci could be distinguished by agglutination tests. These findings were, however, not confirmed by other observers (Neufeld 1903, Aronson 1903, Hasenknopf and Salge 1903); and the problem remained unsolved. In the light of our present knowledge we can hazard the guess that, along these lines alone, it would have remained insoluble. It is true that the renewed interest in the antigenic analysis of the hæmolytic streptococci which followed on the studies of Dochez, Avery and Lancefield (1919) led to the collection of important new facts, and that the results recorded by Bliss (1920, 1922), Gordon (1921), Eagles (1924), and Stevens and Dochez (1926*a* and *b*) went far to confirm and extend the earlier observations of Moser and von Pirquet; but the more detailed studies, which were undertaken before the second world war by Griffith (1926, 1927, 1928, 1934, 1935), Smith (1926, 1927*a*), James (1926), Gunn and Griffith (1928), McLachlan and Mackie (1928) and others (see Chapter 24), revealed a high degree of antigenic heterogeneity among the hæmolytic streptococci, and made it abundantly clear that there was no one antigenic type to which the name *Str. scarlatinae* or *Str. pyogenes* var. *scarlatinae* could be applied, though some types were far more commonly associated with scarlatinal infections than others, just as some types of pneumococci are isolated more frequently than others from cases of lobar pneumonia.

Once it had been shown that *Str. pyogenes* was constantly associated with scarlet fever it was natural to inquire whether this organism would reproduce the disease in any experimental animal. The results obtained when the usual laboratory animals were inoculated with cultures of hæmolytic streptococci, from any source, showed that the guinea-pig, rabbit and mouse differed in their resistance to this bacterial species, and that the type of infection produced varied in relation to the virulence of the particular strain inoculated, and the route of inoculation; but there was no indication of any correlation between a particular type of experimental infection and the source from which the infecting strain was obtained; nor did any of the inoculated animals develop lesions which bore any obvious relation to scarlet fever in man.

Dochez and Sherman (1922), however, found that the subcutaneous inoculation of guinea-pigs with streptococci embedded in agar gave rise to a fever accompanied by an erythematous rash, which later desquamated. Landsteiner, Levaditi and Prasek (1911) produced scarlet fever in monkeys by inoculation of the throat with faucial exudate from scarlatinal patients. *Str. pyogenes* was recovered from the fauces of animals so infected, but these strains failed to reproduce the disease in other monkeys.

The proof that scarlet fever is caused by infection with hæmolytic streptococci rests on a series of observations that have been made on man himself, and these observations have elucidated the pathogenesis of the disease, as well as its causation.

In 1914 Krumwiede, Nicoll and Pratt, a laboratory worker, was accidentally infected by living streptococci. After an incubation of a typical attack of scarlet fever. In 1921 Landsteiner attempted to infect human volunteers by introducing the organism into the mouth a few days after she had attempted to swallow the throat with

typhoid and paratyphoid bacilli, the group of dysentery bacilli, and the cholera and paracholera vibrios. The differences between these groups, however, are differences of respective modal conditions of the differential characters, and at their peripheries the groups merge into one another to give a continuous series of intergrading types whose taxonomic positions and relationships have so far defied precise and satisfactory definition.¹ A useful primary differentiation can be made on the basis of the lactose fermentation which is roughly correlated with pathogenicity; the coliform bacteria ferment this sugar rapidly with the formation of acid and gas in twenty-four hours, while the bacteria of

RELATIONSHIPS OF THE ENTERIC BACILLI (ENTEROBACTERIACEAE)

Escherichææ	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">Escherichia</div> <div style="display: inline-block; vertical-align: middle;">Aerobacter</div> </div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">}</div> </div>	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">coli type—<i>Bacterium (Escherichia) coli</i></div> <div style="display: inline-block; vertical-align: middle;">intermediate types—no species names</div> <div style="display: inline-block; vertical-align: middle;">aerogenes type—<i>Bacterium (Aerobacter) aerogenes</i></div> </div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">}</div>	
	Klebsiella	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">paracolon bacilli</div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">{</div> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">coli type</div> <div style="display: inline-block; vertical-align: middle;">intermediate types</div> <div style="display: inline-block; vertical-align: middle;">aerogenes type</div> </div> </div>	
		<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"><i>Bacterium (Aerobacter) cloacæ</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Bacterium friedlanderii (Klebsiella pneumoniae)*</i></div> </div>	
Proteææ	Proteus . .	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"><i>Proteus vulgaris</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Proteus mirabilis</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Proteus morgani</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Proteus rettgeri</i></div> </div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">{</div> </div>	
Salmonelleææ	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">Salmonella .</div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">{</div> </div>	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"><i>Salmonella typhi</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Salmonella paratyphi A</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Salmonella typhi-murium</i> etc.</div> </div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">{</div> </div>	
	Shigella . .	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;"><i>Shigella shigæ</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Shigella parashigæ</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Shigella flexneri</i></div> <div style="display: inline-block; vertical-align: middle;">Boyd varieties</div> <div style="display: inline-block; vertical-align: middle;">(<i>Shigella boydii</i>)</div> <div style="display: inline-block; vertical-align: middle;"><i>Shigella ambigua (schmitzi)</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Shigella alkalescens</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Shigella sonnei</i></div> <div style="display: inline-block; vertical-align: middle;"><i>Shigella dispar</i></div> </div> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">{</div> </div>	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">Types I to VI</div> <div style="display: inline-block; vertical-align: middle;">Types 170, P 288, D 1</div> </div>
		<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; font-size: 3em;">{</div> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">var. <i>ceylonensis</i></div> <div style="display: inline-block; vertical-align: middle;">var. <i>madambensis</i></div> </div> </div>	

The genera *Erwinia* and *Serratia*, plant pathogens and chromogenic (red) saprophytes respectively, are omitted here though classified with the *Enterobacteriaceae*.

* *Bact. friedländeri* is an upper respiratory rather than intestinal parasite but is closely related to the enteric bacilli.

the other groups, essentially pathogens, do not ferment it. The distinction is not absolute, of course, since both the paracolonic bacilli and certain of the dysentery bacilli are slow lactose fermenters, but is sufficiently marked to have considerable practical value.

Similarly, the dysentery bacilli divide into two groups on the basis of the fermentation of mannitol and are anaerogenic, that is to say, do not produce gas from fermented carbohydrates. While the *Salmonella* group in general produces gaseous fermentations, the typhoid bacillus and *Salmonella gallinarum* are typically anaerogenic and anaerogenic strains of other *Salmonellae*

¹ Because of the lack of better general agreement than now exists regarding the taxonomy of these forms, the writer prefers to retain the generic name *Bacterium* for many of the enteric bacilli. As one worker has put it, a large part of the difficulty in classifying these forms arises from the fact that the "missing links" from a phylogenetic point of view are not missing.

accord with the view that most young children are susceptible to the erythrogenic toxin, but, with advancing years, acquire an active antitoxic immunity, either by passing through an attack of the clinical disease, or, more commonly, by an immunizing infection with a toxin-producing streptococcus without the production of the characteristic scarlatinal syndrome (see Chapter 49 and Zingher 1924).

The administration of larger doses of erythrogenic toxin to susceptible persons yielded even more demonstrative results. In a number of cases such doses induced a generalized reaction with fever, nausea, vomiting and a transient scarlatiniform rash. Most of the recorded instances of this "miniature scarlet fever" were observed during the earlier stages of the study of the erythrogenic toxin; but it still occurs occasionally, when a particularly susceptible person is undergoing immunization with doses of the ordinary size (see p. 1669). This reaction recalls the observation of Gabritschewsky (1907), who, during the inoculation of a large series of persons with a killed culture of scarlatinal streptococci, noted a scarlatiniform reaction in a number of his subjects. It did not occur in persons who had had scarlet fever, nor in persons injected with anti-scarlatinal serum prepared (Moser 1902) by inoculation of horses with scarlatinal streptococci (see also Szirmai 1912).

Additional evidence was contributed by the studies of Trask and Blake (1924), who record the following significant observations. A toxic substance can be demonstrated in the serum of patients acutely ill with scarlet fever that produces a typical skin reaction when injected intracutaneously into persons who have not had scarlet fever, and whose serum gives a negative Schultz-Charlton reaction (*i.e.* contains no antitoxin). Such injections cause no reaction in persons whose serum gives a positive Schultz-Charlton reaction. This toxic substance is not neutralized by a human serum which gives a negative blanching test, but is readily neutralized by a human serum which gives a positive blanching test. It is not neutralized by normal horse serum, but is readily neutralized by a horse anti-scarlatinal serum.

We may also note here that during the course of scarlet fever there is usually evidence of a serum response to other products of *Str. pyogenes*. Recovery is associated with an increase in the resistance of the plasma to streptococcal fibrinolysin (Tillett and Garner 1933, Tillett, Edwards and Garner 1934, Stuart-Harris 1935, Bau and Kleu 1936, Waaler 1936, Gordon and Balteanu 1937) and with an increase in antibody to the O streptolysin of *Str. pyogenes* (Todd 1932, Longcope 1936, Green 1941, Mote and Jones 1941, Rantz, Kirby and Jacobs 1943). Rantz and his colleagues also noted that in 13 of 24 patients with scarlet fever there was a significant rise in agglutinins for the infecting type of streptococcus.

Taking this evidence as a whole, there seems little doubt as to its import. Scarlet fever in man is a disease caused by infection with *Str. pyogenes*. The causative organisms are, for the most part, localized in the throat, though there may be a transient bacteraemia in some cases. The clinical manifestations of the disease result from the action of a soluble toxin, which is absorbed from the local lesion and carried to the susceptible cells and tissues. The varying susceptibility of children and adults, and of the same age groups in different social environments, depends upon the presence or absence of circulating antitoxin. In animals other than man, and perhaps certain anthropoid apes, the disease is not reproduced, even when they are experimentally infected, because of their much greater resistance to the scarlatinal toxin.

Practically all strains of hemolytic streptococci that have been isolated from cases of scarlet fever have been found to belong to Lancefield's Group A (*Streptococcus*

BIOCHEMICAL REACTIONS OF THE ENTERIC BACILLI

Enteric Bacilli			Biochemical Reactions																				
Group	Species	Butt Russell's Slant	Dextrose	Lactose	Sucrose	Mannitol	Dulcitol	Sorbitol	Maltose	Rhamnose	Raffinose	Arabinose	Trehalose	Inositol	Xylose	Sahlin	Milk	H ₂ S	Indol	Gelatin	d-Tartrate	L-Tartrate	m-Tartrate
Coliform Bacilli	<i>Bact. aerogenes</i>	G a	G	G	G	G	G	G	G	G	G	G	G	G	G	G	ac	-	-	-	-	-	-
	<i>Bact. coli</i> var. <i>communior</i>	G a	G	G	G	G	G	G	G	G	G	G	G	-	G	-	ac	-	+	-	-	-	-
	<i>Bact. coli</i> var. <i>communis</i>	G a	G	G	-	G	G	G	G	G	G	G	G	-	G	G	ac	-	+	-	-	-	-
	<i>Bact. cloacae</i>	G ak	G	G	G	G	G	G	G	G	G	G	G	a	G	G	ac	-	+	+	+	+	+
Dysentery Bacilli	<i>Sh. sonnei</i>	a or k	a	a (l)	a (l)	a	-	-	a (l)	a	a	a	a	-	-	a	a(l) ac	-	-	-	-	-	-
	<i>Sh. dyspar</i> var. <i>ceylonensis</i>	a or k	a	a (l)	a (l)	a	a	a	a (l)	a	a	a	-	-	-	a(l) ac	-	+	-	-	-	-	
	<i>Sh. dyspar</i> var. <i>madagascariensis</i>	a or k	a	a (l)	a (l)	a	-	a (l)	a (l)	a	-	a	-	-	-	a(l) ac	-	+	-	-	-	-	
	<i>Sh. shigae</i>	a or k	a	-	-	-	-	-	-	-	a	-	a	-	-	-	ak	-	-	-	-	-	
	<i>Sh. flexneri</i>	a or k	a	-	a	a	-	a	a	-	a	a	a	a	-	a	ak	-	+	-	-	-	
	<i>Sh. ambigua</i> (Schmitz)	a or k	a	-	-	-	-	-	a	-	-	-	-	-	-	-	ak	-	+	-	-	-	
	<i>Sh. alkalescens</i>	a or k	a	-	a	a	a	a	a	a	-	-	-	-	-	a	k	-	+	-	-	-	
	<i>S. typhi</i>	a ak	a	-	-	a	a(l)	a	a	-	a	a	a	-	a	-	ak	+	-	-	+	-	-
	<i>S. gallinarum</i>	a ak	a	-	-	a	a	-	a	a	-	a	a	-	a(l)	-	-	+	-	-	+	+	+
Others	<i>S. pullorum</i>	G ak	G	-	-	G	-	-	G	-	G	G	G	-	G	-	ak	+	-	-	+	+	+
																	ak	-	-	-	+	+	+
																	-	ak	++	-	+	+	+
																	-	ak	++	-	+	+	+
																	-	ak	++	-	+	+	+
	<i>S. paratyphi</i> C (Hirschfeld)	G ak	G	-	-	G	G	G	G	G	-	G	G	-	G	-	ak	++	-	+	+	+	+
	<i>S. cholerae-suis</i> (American)	G ak	G	-	-	G	G	G	G	G	-	-	-	-	G	-	ak	+	-	+	+	+	+
	<i>S. cholerae-suis</i> var.																ak	++	-	+	+	+	+
																	-	ak	++	-	+	+	+
																	-	ak	++	-	+	+	+

G—acid and gas, a—acid, \overline{G} or \overline{a} —most strains ferment, \overline{G} or \overline{a} —most strains do not ferment (l)—late, k—alkaline
 ak—acid to alkaline, c—curd, d—digestion, +- utilization of tartrate, formation of H₂S or indol, or liquefaction of gelatin
 --no action.

ordinary commercial antitoxin should neutralize a high proportion of all toxic strains. Whether an erythrogenic toxin is produced by any other species, or group, of hæmolytic streptococci is as yet uncertain (see Chapter 24).

The Bacteriological Diagnosis of Scarlet Fever.

Scarlet fever, in its characteristic clinical form, is a disease in which a throat infection with *Str. pyogenes* is associated with a rash produced by the erythrogenic toxin, and a syndrome that is, in the main, toxæmic in origin. A diagnosis of a throat infection caused by *Str. pyogenes* is not therefore a diagnosis of scarlet fever; indeed, a clinician will frequently be concerned in distinguishing a throat infection of this type from a case that, because of other criteria, would fall into the scarlet fever category. A throat swab has not, under these conditions, any great diagnostic value. We should expect to isolate *Str. pyogenes* from every case of scarlet fever; but we should also expect to isolate it from many cases to which that diagnosis would not be applicable. It may be noted that the attempt to isolate *Str. pyogenes* from genuine cases of scarlet fever is by no means uniformly successful. In our own experience about 10 per cent. of throat swabs taken during the first three or four days of the disease have failed to yield this organism, though we have almost invariably isolated it during the second half of the first week. As a routine it is advisable to inoculate two blood agar plates and one gentian violet blood agar plate, and to incubate one of the blood agar plates anaerobically in order to favour hæmolysis. Very occasionally, *Str. pyogenes* fails to form hæmolytic colonies, and its recognition becomes particularly difficult (Coburn and Pauli 1941, Colebrook *et al.* 1912). The demonstration of small numbers of streptococci in the throat may be aided by preliminary enrichment in sodium azide crystal violet broth (Pike and Fashena 1946). During convalescence the antistreptolysin content of the blood rises in about 75 per cent. of scarlet fever patients (Keith and Carpenter 1916), but as it also rises in many other forms of streptococcal infection, it is of no specific diagnostic significance.

The Diagnosis of Susceptibility or Immunity to Scarlet Fever.

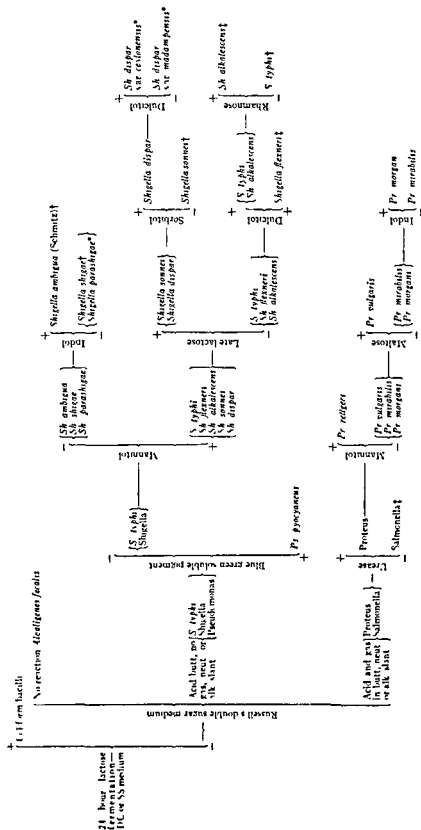
For this purpose we use the Dick test, in which a small dose of toxin is injected into the skin of one arm, and a dose of inactivated toxin into the skin of the other, to serve as a control. The amount of toxin injected is 0.2 ml, containing one skin-test dose, and the method of performing the test is essentially the same as in the Schick reaction (see p. 1576). The following differences should, however, be noted.

The erythrogenic toxin is far more heat resistant than diphtheria toxin. The filtrate that is to serve as a control must therefore be heated at 96° C. for 45 minutes. A control test should never be omitted (see Burton and Weir 1944).

The reaction to the erythrogenic toxin occurs more rapidly than the reaction to diphtheria toxin, and reaches its maximum within 24 hours. The pseudo-reaction, when it occurs, is more rapid than the reaction to diphtheria toxin, and fades more slowly. In this last respect, the time relations of the positive and pseudo-reactions in the Dick test are the reverse of those in the Schick test, and the times of reading the Dick test must be adjusted accordingly.

The interpretation of the test does not differ from that of the Schick reaction. A positive reaction (positive on the test arm and negative on the control) or a

PHYSIOCHEMICAL DIFFERENTIATION OF THE PATHOGENIC ENTERIC BACILLI



Polymultispecifically heterogeneous

immunologically homogeneous, identify by serological methods

subdivided into immunological species or types.

NH 5 type is more rapidly identified by initial culture on bismuth sulfite agar and agglutination in typhoid antiserum

The Epidemiology of Scarlet Fever.

There is every reason to suppose that the epidemiology of scarlet fever is closely analogous to that of diphtheria. In both cases we have diseases characterized by primary throat lesions and a characteristic clinical syndrome that is determined, in the main, by the effects produced by the diffusion of a soluble toxin from the primary lesion. In both, carriers of the causative organism greatly outnumber the clinical cases. In both, the population at large is undergoing, from early childhood onwards, an active immunization resulting from latent or atypical infections (see, for instance, Okell and Parish 1928, Stocks 1930, Allison and Gunn 1932, Okell 1932). They differ, however, in one important respect. Broadly speaking, diphtheria is the only clinical manifestation of *C. diphtheriæ*, whereas scarlet fever is but one manifestation of a wide variety of lesions caused by *Str. pyogenes*.

The earlier observations on the distribution of the causative organism, including those carried out in the authors' laboratory at Manchester and in London (Report 1930, Straker *et al.* 1939), referred to the incidence of hæmolytic streptococci in general, but more recent information is available on the incidence of *Str. pyogenes* in particular. The frequency with which this organism can be isolated from the throat of normal persons not in contact with epidemic streptococcal infection of the respiratory tract varies widely with age, season, country, occupation and doubtless other factors. Thus the following figures may be quoted as examples: healthy children and adults in Melbourne 4.6 per cent. (Keogh *et al.* 1939b); school children in Melbourne 13.3 per cent. (Macdonald *et al.* 1940); students, staff and out-patients in hospital at Patna 10.6 per cent. (Chatterjee and Mitter 1941); parturient women in Shanghai 1.8 per cent., and hospital nurses in Shanghai 4 per cent. (Wu 1941); healthy girls in a residential school in Buckinghamshire 4.2 per cent. (Report 1941); patients suffering from measles on admission to hospital at Bristol 11.7 per cent. (Peters *et al.* 1942); Army recruits in Wyoming 8 per cent. (Schwentker 1943); incoming naval recruits in the United States 4 per cent. (Schwentker *et al.* 1943a); Bantu children 19.0 per cent. (Murray 1943); patients and nurses in the Birmingham Accident Hospital 19.2 per cent. (Williams and Harper 1944); 3.9 per cent. of healthy persons in Denmark (Ernst 1942); 4 per cent. of children admitted to the general medical and surgical wards of the Radcliffe Infirmary, Oxford (Cook *et al.* 1949); the nasal carrier rate in this last group was 0.65 per cent. These figures, it should be pointed out, were obtained mostly by direct plating of the swabs on blood agar; it is possible that, if a preliminary enrichment medium had been used, they would have been higher (see Pike and Fashena 1946).

On the whole, the carrier rate in the throat of normal persons usually seems to vary between 4 and 8 per cent. It tends to be highest in the winter and spring, and lowest in the summer and autumn months.

In any community, such as a school, where tonsillitis or scarlet fever is occurring, even though the number of such cases is small, the incidence of *Str. pyogenes* in the throat tends to be far higher. Carrier rates of 25 or 50 per cent., or even more, are by no means uncommon. In the nose *Str. pyogenes* is much less frequent than in the throat. The normal incidence is below 1 per cent. When it is present in the nose, it is almost invariably found in the throat as well.

We have described on p. 1567 the way in which diphtheria bacilli disappear from the throat during convalescence, and reference to Table 107 will show that about 50 per cent. of diphtheria convalescents have ceased to carry by the 20th day, and 90 per cent. by the 50th day. It has become quite clear that the disappearance of hæmolytic streptococci from the throat during convalescence from scarlet fever is much slower (see Nicholls 1927, Gordon 1927, Gunn and Griffith 1928, Allison and Gunn 1932, Brown and Allison 1935, Allison and Brown 1937, de Waal 1940);

are occasionally met with. The general biochemical groups are indicated in the accompanying table of biochemical reactions.

The separation of the pathogenic enteric bacilli and the related bacteria which may be confused with them on a biochemical basis is of very considerable practical value but in general should be supplemented with, or can be cut short by, serological methods of identification. The specimen is usually cultured on a differential selective medium containing lactose and an indicator together with bile salts to inhibit the growth of contaminants. The biochemical differentiation of the lactose-negative bacteria may be carried out as illustrated in the accompanying dichotomous scheme.

The lactose-fermenting bacteria constitute the subject matter of the present chapter, and the non-lactose-fermenters will be discussed in the chapters immediately following.

BACTERIUM COLI

Bacterium coli (*Escherichia coli*) was described by Escherich in 1886 under the name of *Bacterium coli commune*. The original culture was isolated from the dejecta of a breast-fed infant, and cultures from this source were considered by Escherich to be especially typical. *Bact. coli* is, however, widely distributed, although "ubiquitous" only in the limited sense that it is universally found in the intestinal tract of man and many of the higher animals. It is especially abundant in the colon and is so characteristic an inhabitant of this region of the intestine as fully to deserve the name that has been given it. From fresh, healthy human feces it is often isolated in pure culture by the ordinary aerobic methods, although microscopic examination shows that other kinds of micro-organisms are also present.

Morphology and Staining. The colon bacillus exhibits considerable variation in its morphology. The usual dimensions observed in stained preparations from cultures upon nutrient agar or gelatin range from $2\ \mu$ to $4\ \mu$ in length and from $0.4\ \mu$ to $0.7\ \mu$ in breadth. Very short, oval and coccus-like forms are not infrequently found and usually predominate when the bacillus is observed directly in the normal animal. The bacilli are occasionally observed in pairs or short chains. Some varieties are encapsulated, particularly those found in pathological conditions. Motility is variable, although the most typical strains are motile by peritrichous flagella. Spores are not formed.

Colonies upon nutrient gelatin are more consistent and characteristic in appearance than those upon agar media. They are opaque to partially translucent, smooth, moist and homogeneous in consistency, with entire to undulating edge, and exhibit the maple-leaf appearance common to many of the enteric bacteria (see below). Colonial morphology is somewhat variable upon nutrient agar. Typical colonies are opaque and grayish-white, and there may be a tendency to become a light yellowish brown upon continued incubation. Pigmented varieties are occasionally observed.² In certain differential media the colonies of *Bact. coli* may assume other characteristics typical under the special circumstances. Upon Endo's medium, for example, the colonies are of course, red, but in addition take on a curious metallic sheen that is highly

² Cf. Gililand and Reese Jour. Bact., 1943, 45:499.

outbreak of sore throat or scarlet fever due to the same type of streptococcus in an institution to which he is admitted (Griffith 1931, Moore 1913, Boissard and Fry 1911, Coburn 1911). No bacteriological test is available to distinguish between infective and non-infective strains of *Str. pyogenes*, but it is usually found that persistently infective carriers have some abnormality of the upper respiratory tract, such as a discharge from the nose or ears or enlarged unhealthy tonsils.

The extent to which infection spreads among a population has been studied in institutions (see Griffith 1934, Report 1938, Straker *et al.* 1939, de Waal 1941) and other communities (Schwentker 1913, Schwentker *et al.* 1913a, b). The attack rate is subject to considerable variation, seldom, however, in this country exceeding 10 per cent. and often being very much lower. Though there may be a parallelism between the carrier rate and the attack rate, numerous exceptions occur. From institutional studies we have learned that there may be few cases even in the presence of a high carrier rate, and many cases when the carrier rate is low. Hamburger (1911) and Rubenstein and Foley (1915) had the same experience. This does not seem to be determined wholly or even mainly by the previous streptococcal experience of the children; and as Griffith's studies show, immunity in any case is more or less type-specific. The evidence is far more in favour of Griffith's conception of the existence of strains possessing varying degrees of infectivity and invasiveness (see Stocks 1911). An "epidemic" strain, endowed with both these qualities in a high degree, is liable, when introduced into a given population, to cause a fairly high morbidity, whereas other strains less liberally endowed in these respects will be responsible for merely a few sporadic or "dropping" cases. Analogy with experience of the meningococcus is very close; and there is some experimental evidence to support this conception (Coburn *et al.* 1951). An additional factor that may exert a considerable effect on the extent and severity of scarlet fever is the coincidence of other respiratory disease, such as measles or influenza (see Gordon 1939). According to Stebbins, Ingraham and Reed (1937), who made a very careful study of milk-borne streptococcal outbreaks in New York State, children under 15 years of age are more infective than older children or adults. The secondary attack rate among household contacts was nearly twice as high when the infecting case was a patient less than 15 years old—13.7 as opposed to 7.3 per cent. The same workers quote figures of 20.0 and 5.3 per cent. for children under and over 15 years respectively in a non-milk-borne outbreak in the same State. Gordon (1940) states that children under 5 years are responsible for about eleven times as many secondary cases as are patients aged 15 years or older. Coburn and Paul (1941) likewise found that children were much more infective than adults.

Mode of Spread of Infection.

Though hæmolytic streptococci have been isolated from the desquamated scales of scarlet fever patients (see Allison and Gunn 1932), there is little evidence to suggest that organisms from this source play any appreciable part in the spread of infection. Again, direct infection by droplets or droplet nuclei is probably of minor importance, since very few streptococci are normally expelled from the throat during coughing. Of far greater significance is aerial spread by contaminated dust. Numerous observers have now demonstrated the presence of hæmolytic streptococci in the dust of wards or dormitories housing patients suffering from scarlet fever, tonsillitis or other streptococcal infection (White 1936, Crumckshank and Godber 1939, Stalker *et al.* 1942, Walter and Hucker 1942, Edward 1914; see also Chapter

characteristic when viewed by reflected light. Some varieties are β hemolytic on blood agar, such hemolytic strains occur with much greater frequency in pathological processes than in the normal intestine.

Bact. coli stains readily with the ordinary aniline dyes and is gram-negative. Flagella may be demonstrated by special staining methods

Physiology. The colon bacillus is a facultative anaerobe, growing equally well under aerobic or completely anaerobic conditions. It grows luxuriantly upon the ordinary nutrient media and may be cultivated in synthetic solutions containing an ammonium salt and an organic source of carbon such as glucose. Growth occurs over a temperature range of 10° to 46° C., there is good growth from 20° to 40° C., and the optimum is 37° C.

Milk is curdled with an acid reaction, usually within forty-eight hours. Gelatin is not liquefied, but indol is produced in abundance by cultures in peptone water. Hydrogen sulfide is produced in only very small amounts, cultures in the usual iron or lead acetate media are negative, but more sensitive methods give a positive reaction.

Various sugars are actively fermented with the production of acid and gas, including dextrose, lactose, maltose, arabinose, xylose, rhamnose and mannitol. The fermentation of other sugars, including sucrose, is variable, but the polysaccharides dextrin, starch and glycogen are not fermented. The gas produced is a mixture of carbon dioxide and hydrogen, generally in a ratio of 1:1. The greater part of the acid produced is lactic acid; smaller quantities of formic and acetic acids are formed, together with ethyl alcohol. Succinic acid is also found in variable but small amounts.

The colon bacillus is of ordinary resistance to deleterious influences, being, as a rule, neither as resistant as staphylococci nor as susceptible as the more delicate bacteria. Most strains are killed by exposure to 60° C. for thirty minutes, but occasionally more resistant varieties are encountered. In common with the other gram negative bacteria, it is considerably less susceptible to the bacteriostatic action of dyes than are the gram-positive microorganisms, and selective media containing dyes are commonly used in the primary isolation of the enteric bacteria. The ability of the colon bacillus to grow in the presence of bile is likewise made use of in differential media (MacConkey's broth) in the bacteriological examination of water.

Variation. The existence of two colony types of *Bact. coli* has long been known, the one the flat, maple-leaf form, and the other a smaller, raised, round, moist type. The colon bacillus dissociates into rough and smooth colonial types, the S form giving rise to smooth, round, domed, shiny, translucent colonies, while the R colony type is characterized by an irregular, dull surface, jagged outline and opacity. It has been suggested that the R form is the more virulent.

Toxins. As in the case of many other bacteria, the cell substance is toxic to experimental animals upon parenteral inoculation. The specificity of the endotoxin has not been established. Vincent³ has described two toxic substances formed by the colon bacillus, the one a heat-labile neurotropic toxin and the other a heat-stable enterotropic toxin. An enterotoxigenic substance

³ Vincent: Compt. Rend. Acad. Sci., 1925, 180 239, 407, 1083, 1624, see also Weinberg and Prevot: Compt. Rend. Soc. Biol., 1927, 97.164.

The history of scarlet fever provides an excellent example of an infective disease which has exhibited variations in its behaviour during the past century. We have already noted the mildness of the type of infection now prevalent; but this mildness is of recent development. In 1861-5 the death rate from scarlet fever, in England and Wales, was 982 per million living; twenty years later it had dropped to half this value; in the quinquennium 1921-5 it had fallen to 29 per million, and in that of 1947-51 to less than 1 per million. This decrease in mortality has, during recent years, been associated with a shift in the age incidence of the disease, so that the maximum morbidity now occurs in the 5-10 age group.

Prophylaxis

In a disease such as scarlet fever in which the carriers among the general population largely outnumber the cases we should not expect isolation in fever hospitals to exert any appreciable effect on the frequency of the disease among the population at large; and we may note that Woods (1928), in a statistical study of the incidence of scarlet fever in various districts in this country over a period of 25 years, could find no evidence that isolation had any effect on the prevalence of the disease.

So far as the patient himself is concerned, it must be remembered that cross-infections in hospitals are very common, and that they may be responsible for complications and for increasing the duration of treatment. Their incidence may be greatly reduced by nursing the patients in cubicle blocks, or in wards reserved for patients infected with the same serological type of organism (Allison and Brown 1937, Cooke and Neisser 1937, Peters *et al.* 1942, Bergman 1944), though the administrative difficulties of the latter method are often considerable. A further measure that promises success is the treatment of floors and bed clothes with dust-laying oils (see Wright *et al.* 1944, Harwood *et al.* 1944, Report 1944, and Chapter 91). In order to diminish the risk of cross-infections, it is wise, if possible, to isolate the patient at home; or, if admission to a hospital is advisable, to restrict the stay to the minimum period necessary—usually 2 to 3 weeks (see Gordon 1940).

Chemoprophylaxis of persons exposed to risk was tried on a large scale in American naval depots during the second world war. Watson and his colleagues (1943), for example, recorded apparent success from the daily administration of 1 gm. of sulphadiazine to contacts during an outbreak of scarlet fever. Further experience, however, of this method proved disappointing owing to the emergence of sulphonamide-resistant strains—particularly Types 17 and 19—which not only gave rise to fresh cases during the prophylactic régime, but proved unsusceptible to treatment by therapeutic doses (see Report 1945, Damrosch 1946, Delamater *et al.* 1946).

Outbreaks of scarlet fever in schools and other institutions are often very difficult to deal with. Segregation of heavily infected carriers and the passive immunization of contacts may at times be called for, but our control over this disease cannot be compared as yet with the ease with which we can bring outbreaks of diphtheria to a sudden close. Particular care should be taken before admitting convalescents to an institution. Though most convalescent carriers appear to become non-infective within 3 or 4 weeks, occasional carriers may remain infective for much longer and be responsible for introducing the disease into the community. Search should be made in all convalescents for abnormality of the upper respiratory passages, and any child who is found to be a carrier of *Str. pyogenes* and to

that has been reported by other workers⁴ is of interest in connection with the epidemiological implication of colon bacilli in outbreaks of food poisoning. Some strains of *coli* produce filterable hemolysin.

Pathogenicity for Man. The ability of the colon bacillus to set up pathologic processes in man is very slight. The microorganism is constantly present in the intestinal tract and, although excessive sugar fermentation with the liberation of irritant acids and gas may possibly be responsible for some cases of diarrhea, in general it does little harm. The term pathogenicity is, as has been pointed out elsewhere, a relative one, and *Bact. coli* may, on occasion, invade the body tissues and set up a focus of infection. The urinary tract is probably the most frequently invaded, and the majority of cases of cystitis are of *coli* etiology. The colon bacilli may also play a part in the formation of gallstones, the bacillus is frequently found in the core of gallstones and, in culture, can precipitate cholesterol and other biliary constituents. The injection of colon bacilli into the healthy urinary bladder or gallbladder does not produce infection in experimental animals, but infection occurs if the bile duct or urethra is obstructed. Local infections such as abscesses, conjunctivitis and the like have been observed but are not common. *Bact. coli* septicemia is very rare, but may occur as an agonal invasion in acute infective processes. A hemorrhagic septicemia caused by *Bact. coli* sometimes occurs in newborn children and is known as Winckel's disease.

In general, however, *coli* infection is rare except in cystitis, and it is probable that the pathogenic powers of the colon bacillus have been exaggerated, particularly by the early writers who in many instances failed to differentiate *coli* from other members of the enteric group. It may be noted that the common occurrence of agonal or postmortem invasion of the body by the colon bacillus tends to diminish the value of evidence derived from finding this bacterium in the internal organs after death.

Pathogenicity for Lower Animals. *Bact. coli* is of low pathogenicity for laboratory animals. Two milliliters of a broth culture injected intraperitoneally will kill a guinea pig within a few days, and killed bacilli are very nearly as effective. Spontaneous infection of lower animals is not common. The diarrhea of young calves known as "scours" has been attributed to *Bact. coli* septicemia. The colon bacillus is also thought to be a factor in the causation of diarrhea of foals and young pigeons. Local infection may occur; in one series of 286 cases of bovine mastitis, 10 were apparently due to members of the colon-aerogenes group, of which 3 were typical *Bact. coli*.⁵

Varieties of Bacterium Coli. The above discussion has been one of "typical" *Bact. coli* and, although the description holds true in general, "atypical" strains are not infrequently found. These varieties may be separated, for pedagogical purposes, into two groups, one "fermentatively atypical," and the other, the strains intermediate between *Bact. coli* and *Bact. aerogenes*. The latter straddle the lines of demarcation drawn between the two species by various differential tests and will be discussed in a later section in that connection.

The Fermentative Varieties. These may, of course, be many, owing to

⁴ Jordan and Burrows Jour. Inf. Dis., 1935, 57:121.

⁵ Gwatkin, LeGard and Hadwen Canadian Jour. Comp. Med., 1938, 2:155.

As in diphtheria immunization, the most significant results have been reported from fever hospitals or other institutions in which the risk of contracting scarlet fever is unusually high.

Benson (1928) records the effect of Dick-testing and immunization on the incidence of scarlet fever among the nursing staff of the Edinburgh City Fever Hospital. From 1919 to 1925, the incidence of scarlet fever fluctuated between 4·82 and 10·27 per cent. Immunization was started in October 1925; in 1926 the incidence was 3·52 per cent. and in 1927 it was 0·67 per cent. The figures for 1928-31, as given in the Annual Reports of the Medical Officer of Health for Edinburgh, show that the very low incidence of scarlet fever was maintained.

Dick and Dick (1929), summarizing their records, state that among 11,584 immunized persons in institutions where scarlet fever was epidemic, no case of scarlet fever occurred, and that of 1,191 nurses or interns immunized before commencing work in fever hospitals none contracted the disease.

In a group of nurses aged 18-25 years, Cruickshank (1936) found the percentage incidence of scarlet fever to be 0·0 in the naturally Dick-negative, 2·8 in nurses Dick-negative by immunization, 15·0 in nurses who were positive after immunization, and 25·0 in nurses who were originally positive but were not immunized.

It may be noted that the experience of active immunization in man is in general accord with the view that the protection afforded is less effective against the invasive than against the toxæmic effects of hæmolytic streptococcal infection. Thus Kinloch and others (1927) note an increase in the frequency of streptococcal tonsillitis among the nursing staff of a fever hospital after immunization, suggesting that the typical scarlatinal syndrome had been replaced by an infection confined to the throat. Benson (1928), on the other hand, recorded a slight diminution in the incidence of tonsillitis among the nursing staff after immunization; but the diminution was insignificant in comparison with the much greater decrease in the incidence of scarlet fever among the staff over the same period (see also Place 1933, Rhoads 1949).

In practice, owing partly to the difficulty of giving five injections, partly to the severe clinical reactions that follow in some subjects, and partly to the absence of protection conferred against infection as distinct from toxæmia, the use of active immunization is very limited. Its chief value is for nurses in fever hospitals, for some children's institutions, and for countries in which the death rate from toxic scarlet fever is high.

Inoculation of contacts with *antitoxic serum* affords passive protection for 2 to 3 weeks, and may be used in sick or debilitated children or for other special reasons, when the risk of repeated exposure is negligible.

The Treatment of Scarlet Fever.

Antitoxic Sera.—Clearly, the demonstration that a high-titre antitoxic serum can be obtained by immunizing a horse against erythrogenic toxin raises the question of the value of such antisera in the treatment of the disease in man.

It seems reasonable to suppose that a specific antitoxic serum will exert an effect on those manifestations of scarlet fever that are directly toxic in origin. We might expect a saving of life in severe toxæmic cases, a diminution in the rash and a more rapid subsidence of the fever in the milder type of infection. Whether the serum exerts an indirect effect on the bacterial invasion, by lifting the burden of toxæmia from the body and thereby improving its antibacterial defences, it is difficult to say.

the number of sugars whose fermentation by these bacteria is variable. A few of these are, however, well known and may be mentioned briefly. In regard to the sucrose fermentation, about half of the strains of *coli* isolated are able to ferment this disaccharide while the other half are not, and the two fermentative types have been given different names, the strains of *Bact. coli* that ferment sucrose are called *Bacterium coli communior*, while those that do not ferment sucrose are termed *Bacterium coli communis* (or *commune*).

Much less frequently strains of *Bact. coli* are isolated which do not immediately ferment lactose, i.e., their colonies on Endo's medium are white. In the course of incubation, however, red papillae develop on the white colonies which, upon subculture, ferment lactose and breed true. Such strains have been known for many years and are termed *Bacterium coli mutabile*. This phenomenon is discussed elsewhere (p. 174) in connection with bacterial variation.

A fourth fermentative type which resembles *Bact. coli* in all particulars except that it ferments sugars with the production of acid and no gas is generally known as *Bacterium coli anaerogenes*. Although these names are commonly used they have no taxonomic standing and are a matter of convenience only.

The Paracolon Bacilli. Another fermentative variety whose relationship to the typical coliform bacteria is not clear is that which is characterized by late (five to fourteen days) lactose fermentation. In this respect these strains resemble the Sonne and dysenter dysentery bacilli, and some strains resemble the typhoid and dysentery bacilli in that they are anaerogenic. They may be grouped into paracolon coli, -intermediate, and -aerogenes strains.⁶ They appear to be pathogenic to a minor degree, not only having been found in association with mild enteric infection, but also having produced laboratory infections. Perhaps the greatest difficulty in assessing the possible pathogenicity of the paracolon bacilli is that of identifying types and strains with precision. In a number of instances, however, immunologically identical or closely related strains have been found in outbreaks of diarrheal disease, and the close correspondence of the immunological character of the microorganism with the epidemiology of the disease is strongly suggestive of an etiologic relationship. They are perhaps to be regarded as transitional forms between the lactose-fermenters and the non-lactose-fermenters and possibly allied to the Sonne dysentery bacilli. The group is, however, a heterogeneous one and is not to be regarded as a well-defined type of enteric bacteria.

Bacterium cloacae, regarded by many as a distinct species, may be mentioned here, for it differs from *Bact. coli* in that indol formation is variable and gelatin is liquefied. The latter property serves to set *Bact. cloacae* off from the other members of the group. It is found as an intermediate type which has not been found to have some pathogenic properties.⁷

⁶ See Stuart, Wheeler, Rustigan and Zimmerman Jour. Bact., 1943, 45:101; Stuart and Rustigan Amer. Jour. Pub. Health, 1943, 33:1323.

⁷ Caminita et al.: Pub. Health Repts., 1943, 58:1165.

streptococci. In practice, it is advisable to give antiserum when the initial toxæmia, as shown by a temperature of 102° F. or over and a bright rash, is severe. A single dose of 3,000-6,000 U.S.A. units should be injected intramuscularly (Joe 1951). Though the antiserum may protect to some extent against complications, there is little evidence of any curative effect when it is given after the complications have developed.

The Standardization of Scarletinal Antitoxin.—As in the case of all other antisera, the standard of reference is an arbitrarily selected sample of serum. The unit was originally defined as an amount of antitoxin neutralizing a certain number of "skin test doses" in man (see Dyer 1928), but later the proper course was taken (see Chapter 43) of equating the unit to the specific activity of an arbitrarily selected quantity of a standard preparation of antitoxin. For many years a standard antitoxin and an antitoxic unit, established by the National Institutes of Health (N.I.H.) in the United States of America, had the status of a provisional international standard and unit. In 1953 a stronger antitoxin was established as the international standard, with an international unitage equivalent to the existing N.I.H. unit (Report 1953).

The neutralizing power of the antitoxin may be estimated by intracutaneous titration of toxin-antitoxin mixtures, or by a flocculation test. Most laboratory animals, however, are insusceptible to the toxin. Parish and Okell (1927) attempted to circumvent this difficulty by testing the capacity of antitoxins to prevent acute septicæmic death in rabbits inoculated with virulent *Str. pyogenes* (see also O'Brien *et al.* 1929).

In 1930, however, Fraser and Plummer described a method of titrating scarlatinal toxin and antitoxin in the skin of Chinchilla rabbits; and further experience with this test suggests that, with certain modifications, it gives results as accurate as those obtained in the human skin, or by the method of Parish and Okell (see Kolchin *et al.* 1933, Plummer 1934, Buttle and Lowdon 1935). A version of Buttle and Lowdon's method is accepted as satisfactory in the British Pharmacopœia (1953). Veldee (1932) and Malcolm and Wyman (1935) developed a similar test, using the ear of the rabbit; but the method is difficult to apply except by experienced workers and with rabbits selected for susceptibility.

Many attempts have been made to titrate the erythrotoxin by a constant-antigen flocculation method analogous to the Ramon titration of diphtheria toxin. Successful *in vitro* titrations of erythrogenic toxin, tallying with those in man and animals, have been made, using solutions of toxin containing over 50,000 skin test doses (Rane and Wyman 1937, Bunney and Koerber 1941, Hottle and Pappenheimer 1941, Proom 1941, Rao and Moloney 1950).

Convalescent Serum.—Convalescent serum in the treatment of scarlet fever has certain advantages over horse antitoxic serum and is preferred by some workers (see Gordon 1933, Fox 1937, Platou, Dwan and Hoyt 1941). It does not give rise to serum reactions, it is homologous and is therefore excreted more slowly, and it may have antibacterial as well as antitoxic properties. Large-scale tests of pooled convalescent serum indicate that it may be at least as effective as antitoxin in reducing both mortality and complication rates (Fox and Hardgrove 1937). Top and Young (1939, 1941), who recommend its use in the septic forms of scarlet fever, found that a pooled serum containing 60 antitoxic units per ml. was as effective as a horse antitoxin containing 6,000 units per ml., and concluded that convalescent serum owed its efficacy to antibacterial as well as to antitoxic antibodies. Moore and Thalheimer (1939) tested convalescent sera for antitoxic and antibacterial activity. The antitoxin content averaged 3.3 units per ml. e bactericidal power,

BACTERIUM AEROGENES

Escherich originally described two types of gram-negative lactose-fermenting bacilli, the one *Bact. coli* and a second which was non-motile, somewhat shorter and plumper, and which clotted milk more rapidly. This second variety, first termed *Bacterium lactis aerogenes*, is now generally known as *Bacterium aerogenes* (*Aerobacter aerogenes*). *Bact. aerogenes* is commonly found in soured milk and, unlike *Bact. coli*, appears to live a saprophytic existence in nature on the surface of grains and similar places. Because of the apparent difference in the origin of these two kinds of lactose-fermenters, their differentiation has been a matter of great interest in connection with the use of *Bact. coli* as an indicator of fecal pollution (p. 253).

Because of the close resemblance of *Bact. coli* and *Bact. aerogenes*, the latter may be adequately described in terms of its differences from the former. The morphological differences are minor and variable and cannot be used



Fig. 66. *Bacterium aerogenes*. Smear from a pure culture. Note the coccobacillary form. Fuchsin, $\times 1050$.

as differential characters. *Bact. aerogenes*, it may be noted, is often encapsulated, while the presence of capsules on *Bact. coli* is relatively infrequent. In general, the fermentative power of *Bact. aerogenes* is somewhat greater than that of *Bact. coli*, the ratio of carbon dioxide to hydrogen in the gas produced is about 2:1, milk is curdled more rapidly, and starch is fermented. Growth in gelatin is more luxuriant, in gelatin tubes a projecting "nail-head" growth is characteristically produced. Indol is not produced in peptone solution.

In conjunction with the above differences, three additional tests are commonly used in the differentiation of these bacteria:

(1) The methyl red test is no more than the determination of the pH of a dextrose broth culture after two to four days' incubation. *Bact. coli* produces and maintains a high acidity, and when the indicator is added it is red and *Bact. coli* is said to be methyl red positive. In *Bact. aerogenes* cultures, on the other hand, the hydrogen ion concentration is lower, the added indicator is yellow, and *Bact. aerogenes* is said to be methyl red negative.

response to infection. In some, the prevailing clinical disease was scarlatina; in others, it was sore throat, without the rest of the scarlatinal syndrome (Davis and Rosenow 1912, Stokes and Hachtel 1912, Coleman and Wheeler 1926). This epidemiological evidence has been greatly strengthened during recent years by the studies of Griffith and others (see Glover and Griffith 1931, Griffith 1934) which have shown that a single antigenic type of *Str. pyogenes*, spreading in a closed community such as a school, will produce scarlet fever in some cases and tonsillitis in others. It seems probable that the alternative clinical manifestations of tonsillitis and scarlet fever are determined mainly by the ratio between the toxigenicity of the infecting strain, and the antitoxic immunity of the host (see Okell and Parish 1928, Okell 1932).

Acceptance of this view entails the assumption that different strains of *Str. pyogenes* vary considerably in their ability to produce the erythrogenic toxin *in vivo*, since our knowledge of the distribution of antitoxic immunity, as revealed by the Dick test, renders it improbable that the average resistance of the communities affected can have differed widely enough to account for the striking difference in the clinical manifestations observed.

It would perhaps be unwise to interpret the term "toxigenicity" too literally, because the strains may also be characterized by differences in invasiveness and in the capacity to multiply freely in the tissues. The studies of Griffith and his colleagues on outbreaks of streptococcal infection in schools supply some of the strongest evidence in support of the view that there exist "epidemic strains" endowed with a heightened power of spreading naturally among the population at risk. On the whole strains of *Str. pyogenes* giving rise to scarlet fever appear to be more pathogenic than those giving rise only to tonsillitis. Rhoads (1949), for example, observed among nurses at Cook County Hospital an incidence of otitis media twice as high and of nephritis five times as high in cases of scarlet fever as in cases of ordinary streptococcal sore throat. A good deal of unpublished evidence points in the same direction, but further observations on this point are called for.

It is by no means clear what proportion of sore throats met with in practice is due to infection with *Str. pyogenes*. In the study carried out at Glasgow by Anderson and his colleagues (Landsman *et al.* 1951) the observers were surprised to find that only 68 out of 100 sore throats yielded hæmolytic streptococci on culture of the nose or throat. Nor is it clear, in many cases of sore throat in which *Str. pyogenes* is found, what part this organism is playing in the causation of the lesions. In the study just quoted, the group of 46 cases in which hæmolytic streptococci were present in abundance contained a higher proportion of cases exhibiting headache, anorexia, vomiting, sweating, chills and rigors than the group of 22 cases in which streptococci were scanty or the group of 32 cases in which they were absent. In these two latter groups there was a higher proportion of cases with sneezing, rhinorrhœa, stuffy nose and cough. The authors are on safe ground when they conclude that a clinical diagnosis of streptococcal sore throat cannot be made with any degree of assurance.

Mention has already been made under scarlet fever of the occasional prolonged infectivity of convalescent carriers having enlarged unhealthy tonsils. In some of these, throat swabs may fail to detect hæmolytic streptococci. *Str. pyogenes* can usually be isolated from about 50 per cent. of excised tonsils (Elliott 1939, Keogh *et al.* 1939b, Macdonald *et al.* 1940). Rantz (1941) records figures of 32·7 per cent. from the tonsils after removal, but of only 16·4 per cent. from throat swabs before tonsillectomy.

(2) The *Voges-Proskauer reaction* is a qualitative test for the presence of acetylmethylcarbinol or, more properly, for diacetyl. In the fermentation of glucose *Bact. aerogenes* forms acetylmethylcarbinol along with organic acids and other products, while *Bact. coli* does not form this substance. The culture is grown in glucose-peptone broth for two to four days. When 5 ml. of 10 per cent potassium hydroxide solution is added a deep pink color develops on standing in cultures of *Bact. aerogenes* but not in cultures of *Bact. coli*. *Bact. aerogenes* is, then, Voges-Proskauer positive and *Bact. coli* is negative and there is a negative correlation between the Voges-Proskauer test and the methyl red test. The mechanism of the Voges-Proskauer test is as follows: upon standing in the presence of alkali the acetylmethylcarbinol present is oxidized to diacetyl, which in turn combines with an unknown constituent of the peptone to form the colored substance. Acetylmethylcarbinol is not formed during the early stages of fermentation by *Bact. aerogenes* but as a consequence of further decomposition of the initial products of fermentation, hence a two- to four day culture must be used.

(3) The *citrate test* is dependent upon the ability of *Bact. aerogenes* to utilize sodium citrate as a sole source of carbon in a synthetic medium. A medium consisting of sodium ammonium phosphate, potassium phosphate, magnesium sulfate and sodium citrate is inoculated with the bacteria. *Bact. aerogenes* grows in this solution and is said to be citrate-positive; *Bact. coli* does not grow to any appreciable extent and is said to be citrate-negative.

The differential reactions of *Bact. coli* and *Bact. aerogenes* may be summarized in tabular form:

	CO ₂ H ₂	Indol	M R	V P	Citrate
<i>Bact. coli</i>	1 1	+	+	-	-
<i>Bact. aerogenes</i>	2 1	-	-	+	+

The four most commonly used tests, indol, methyl red, Voges-Proskauer and citrate, are frequently referred to by the mnemonic "Imvic" or "IMViC," which fixes them in order.⁸ In this terminology typical *coli* is (+ + - -) and typical *aerogenes* (- - + +).

Other criteria have been used from time to time, including the fermentation of cellobiose, inositol, glycerol and other carbohydrates, the utilization of uric acid and the Eijkman test. The last consists in the fermentation of lactose at $45.5^{\circ} \pm 0.2^{\circ} \text{C}$., and gas formation in forty-eight hours is taken as positive. *Bact. coli* is positive to this test, while *Bact. aerogenes* is negative.

The Colon-Aerogenes Intermediates. "Typical" *Bact. coli* and "typical" *Bact. aerogenes* are, as indicated above, readily differentiated, but these bacteria represent extremes which are connected by a variety of intergrading forms. On the basis of the IMViC tests 16 combinations are possible, and all of these

⁸ Cf. Parr, Amer. Jour. Pub. Health, 1936, 26:39. The relationships of the coliform bacteria are discussed at length in the review by Parr, Bact. Rev., 1939, 3:1. See also Vaughn and Levine: Jour. Bact., 1942, 44:487.

Committee 1925, Fitzgibbon and Bigger 1925, Colebrook, L., 1926, Kinloch *et al.* 1928, Colebrook, Dora C., 1935, and others). Dora Colebrook (1935), for instance, in reviewing the results recorded by various workers, notes that among the fatal cases of puerperal fever the proportion due to infection with hæmolytic streptococci varied from 68 to 96 per cent. Most of these streptococci belong to Group A, a small proportion to Groups B, C, D and G, and very rarely to some of the remaining groups (Lancefield and Hare 1935, Gardner 1939, Chatterjee and Mitter 1941). Infection by hæmolytic streptococci other than Group A is not usually severe, though among these milder infections a number of authors have noted severe and sometimes fatal cases of septicæmia, often associated with infective endocarditis, following puerperal or post-abortion infection by streptococci of Groups B, G and rarely C (Colebrook and Purdie 1937, Fry 1938, Macdonald 1939, Hill and Butler 1940, Rosenthal and Stone 1940, Ramsay and Gillespie 1941).

Puerperal fever results from streptococcal invasion of the tissues. There is no reason to suppose that the erythrogenic toxin plays any essential part in its causation. The disease is as common in women with a negative Dick reaction as in those who react positively (Burt-White *et al.* 1930, Stent 1930, Baird and Cruickshank 1930); and an attack of the disease in a Dick-positive woman does not render her Dick-negative (Kenny and Colebrook 1937).

Sources of Infection.—The question to be decided is whether the streptococci are of intrinsic or extrinsic origin—whether, that is to say, they are present in the genital tract before labour or are introduced from without during labour or the puerperium. The balance of evidence strongly favours their extrinsic origin.

Careful studies of the vaginal flora before labour have shown that hæmolytic streptococci are very infrequent (Fromme 1908, Kanter and Pilot 1924, Lockhart 1925), and that the small proportion of strains that are found then or during the normal puerperium do not usually belong to Group A (Hare and Colebrook 1934, Lancefield and Hare 1935). Nor can *Str. pyogenes* be demonstrated on the perineal or peri-anal skin of normal pregnant women (Colebrook, Maxted and Johns 1935) or in their faeces during the early stage of labour (Hare and Maxted 1935).

If we abandon therefore the intrinsic origin of infection, we must determine the source of the streptococci that reach the genital passages from without. The possibilities are the nose and throat of the patient herself or of some other person in the vicinity. Since the normal nasopharyngeal carrier rate of *Str. pyogenes* is about 6 per cent., it would be unjustifiable to conclude that a strain isolated from the vagina was necessarily derived from the throat of the patient or a contact; but by identifying the specific type of each of the streptococci isolated, far more significance can be attached to the results.

Smith (1931, 1933), studying 49 cases of puerperal fever caused by *Str. pyogenes*, found an antigenically identical strain in the throat or nose of 8 of the patients and of 31 of the contacts, thus accounting for 39 out of 49 of the series. Dora Colebrook (1933), studying 63 cases, obtained corresponding figures of 24 and 39 respectively, though in some of the latter group the organism was isolated from the nasopharynx of both the contact and the patient herself, rendering it difficult to decide in which direction the transfer had occurred.

We may conclude that in a fairly high proportion of cases the probable source of uterine infection with *Str. pyogenes* during labour or the puerperium is the throat or nose of some person in contact with the patient—usually a doctor, midwife, or nurse—or of the patient's own throat or nose.

have been found.⁹ The allocation of these intermediate forms to *coli* or *aerogenes* is, obviously, a difficult matter and probably not advisable. It is the practice of many to set up a "*coli* group," an "*aerogenes* group" and an "intermediate group" which may be referred to in the aggregate as the *coliform bacteria*. The existence of the intergrading types so lumped and the atypical strains of *coli* discussed earlier has interesting phylogenetic implications that cannot be considered here.

The Immunological Relationships of the Coliform Bacteria. The antigenic structure of the colon bacilli has been investigated by a number of workers and most intensively by Kauffmann.¹⁰ There appear to be three kinds of antigens present, viz.:

- (1) Heat-stable O antigens, of which 110 have been found. Of these, 25 occur with sufficient frequency for diagnostic use with most strains of coliform bacteria.
- (2) Somatic surface antigens, designated as "envelope" antigens or K antigens. These function as "blocking antigens" in that their presence interferes with agglutination with O antisera. Three kinds of K antigens have been described.
 - (a) Those which are designated L antigens are thermolabile and the O agglutinability of bacterial suspensions is restored by boiling. L antisera may be prepared by absorption of LO sera with the homologous O antigen, viz., boiled bacteria. The colonies of strains containing L antigens are somewhat more opaque than those which do not contain them. About 24 L antigens have been described.
 - (b) The component of the K antigen designated the A antigen is present in encapsulated coliform bacilli, is a specific polysaccharide, and bacteria containing it give a *Quellung* reaction in antiserum. It differs from the L antigen in that it is thermostable. So-called N variants lacking the antigen are found in translucent areas at the edge of the large, dense and relatively opaque colony. About 20 A antigens have been found.
 - (c) An antigenic component of the K antigen complex, designated the B antigen, is thermolabile but differs from the L antigen in that the heated antigen can absorb antibody though heated suspensions will not agglutinate in mono specific B antiserum. The B antigen appears to be relatively rare.
- (3) The flagellar or H antigens of the coliform bacilli are often poorly developed. Some 22 components have been found of which 20 are used for purposes of identification.

Kauffmann has developed a serological classification of coliform bacilli, largely *Bact. coli*, based on the distribution of O, K and H antigens. In general, about 80 per cent of strains having K antigens contain L antigens, and the other 20 per cent contain A antigen or B antigen. Strains containing K antigen appear in a general way to be the more toxic and more resistant to phagocytosis and the bactericidal action of antibody, and are found more frequently in pathological material than in feces. Some of the coliform bacteria are immunologically related to some of the other gram-negative bacilli, such as the plant pathogens, Friedlander's bacillus and Salmonella, while others appear to be related to certain of the pneumococcus types.

Wallick and Stuart¹¹ have made the interesting observation that while most of the colon bacilli harbored by a given individual were immunologically identical, or nearly so, individuals showed a continuous succession of types, each predominating for a few weeks or months and then being replaced by a

⁹ Sanborn Jour. Bact., 1944, 48:211.

¹⁰ See the review by Kauffmann Jour. Immunol., 1947, 57:71.

¹¹ Wallick and Stuart Jour. Bact., 1943, 45:121.

of the female genital tract; and the observations of White (1933) suggest that this may be their principal natural habitat. In this case therefore there can be little doubt that puerperal infection is largely intrinsic. (For other references in relation to anaerobic streptococci and their rôle in puerperal fever, see Chapter 24.)

Other Types of Puerperal Infection.—It remains to add that a few cases have been reported in which a fatal pyæmic infection due to *Staph. aureus* has developed during the puerperium; but these are rarities.

In regard to the less severe and fatal types of puerperal infection, which are for the most part localized to the pelvis, a variety of bacteria may be responsible. Many cases are due to a localized infection with *Bact. coli*, and these are not infrequently associated with an infection of the urinary tract. Another group of cases are due to pelvic infections with the gonococcus; and another group, especially after abortion, to infections with *Cl. welchii* (see Butler 1941). Infections caused by anaerobic non-sporing bacilli of the *Fusiformis* group are briefly considered in Chapter 79.

Other Infections associated with Hæmolytic Streptococci

Cellulitis and other forms of suppuration due to *Str. pyogenes* are discussed in Chapter 67; the part played by this organism in acute respiratory infections is considered in Chapter 74, and its relation to acute rheumatism in Chapter 68.

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fresh type. It was noted also that immunologically identical types were sometimes biochemically different

The Ecology of the Coliform Bacteria. The distribution of the coliform bacteria in nature is a matter of some importance in connection with the use of these organisms as indicators of fecal pollution and has been studied by a number of workers. The results of these studies are most conveniently expressed in the accompanying tabular form modified from Griffin and Stuart.¹² Although the percentages given there may not be regarded as an accurate expression of the distribution of the coliform bacteria in nature, it will be

PERCENTAGE DISTRIBUTION OF COLIFORM TYPES*

Source	Coli	Aer.	Int	Irr	Total
Milk	28.8	49.5	20.3	1.4	2224
Water	51.6	28.6	18.5	1.3	9496
Soil	23.8	54.3	18.8	3.1	1330
Grains	17.9	73.8	7.3	1.0	587
Feces	87.9	5.2	6.8	0.1	3974

* As compiled from various authors. In many instances the strains studied were not isolated at random; the percentages are, therefore, weighted, and great significance cannot be attached to them.

clear that no strict lines of demarcation regarding habitat may be drawn. Aerogenes and intermediate types are found in the intestinal tract in appreciable proportions, but it is probable that *coli* found in water, soil and elsewhere in nature is present as contamination and is always fecal in origin.

FRIEDLANDER'S BACILLUS

A heavily capsulated bacterium, closely related to the coliform bacilli,¹³ was described by Friedländer in 1883 as the causative agent of pneumonia. As indicated in a previous chapter, the pneumococcus is the causative bacterium in the great majority of lobar pneumonias, but the bacillus described by Friedländer is also responsible for a small proportion of pneumonias in man. The bacterium has been variously known as the *pneumobacillus*, *Bacterium pneumoniae*, *Bacterium friedlanderii*, *Bacillus mucosus capsulatus*, *Encapsulatus pneumoniae* and *Klebsiella pneumoniae*.

Morphology and Staining. Friedländer's bacillus is a short, thick oval rod 1 to 2 μ long and 0.5 to 0.8 μ thick, arranged singly and in pairs end to end. The diplobacillus arrangement is common in the body. Some strains are pleomorphic in culture, showing filaments and other forms, but these are not usual. These bacteria are non-motile and non-spore forming. A heavy capsule

¹² Griffin and Stuart. Jour. Bact., 1940, 40:83.

¹³ See the comparative study by Osterman and Rettger. Jour. Bact., 1941, 42:699.

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is present on the bacilli in the body and is particularly evident in cultures on the richer media such as blood agar or media containing sugar.

A slimy growth is produced on artificial media, and the colonies are round, amorphous, raised with a glistening surface and mucoid in consistency. There is no hemolysis on blood agar.

The Friedländer bacilli stain readily with the usual aniline dyes, and are gram-negative. The capsule may be demonstrated in stained smears by special methods.

Physiology. These bacteria are facultative anaerobes whose optimum temperature is 37°C . and temperature growth range from 12° to 43°C . Nutritive requirements are exceedingly simple; these microorganisms grow luxuriantly upon the ordinary nutrient media and in simple synthetic solutions. In the writer's laboratory, representatives of all the types of Friedländer's bacillus have been found to grow well in the presence of an ammonium salt and simple carbon compounds such as acetate and, in one instance, formate.

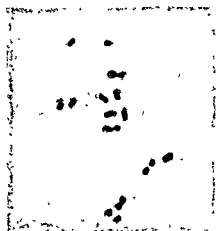


Fig 67. Friedländer's pneumobacillus showing the capsules, blood-agar culture; fixed with methyl alcohol and stained with carbol gentian violet (Wherry).

Fermentation reactions are highly variable from strain to strain, but in general a number of sugars are fermented. The lactose fermentation is variable. Gelatin is not liquefied and indol production is variable. In view of the relation of these bacteria to the coliform group it may be noted that the methyl red and citrate tests are positive, while the Voges-Proskauer test is negative.

The Friedländer bacilli are killed by exposure to 55°C . for thirty minutes but are unusually resistant to drying and are said to survive for a period of months. They are of average resistance to the usual germicidal chemicals.

Variation. These bacteria dissociate as rough and smooth forms. The commonly observed colony type is regarded as the smooth form although analogous to the "mucoid" phase observed in some other bacteria. As in the case of the pneumococcus, the S-R transformation is associated with the loss of the capsule and with a loss of immunological type specificity. The smooth form is virulent and the rough form avirulent.

Classification. Although an inhabitant of the upper respiratory rather than intestinal tract, Friedländer's bacillus is closely related to the coliform bacteria, especially *Bact. aerogenes*. In the Bergey (1948) classification it is

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grouped with *Bact. coli* and *Bact. aerogenes* under the Escherichiae as *Klebsiella pneumoniae*. This bacterium is, however, exceedingly difficult to differentiate from the mucoid phases of the coliform bacteria and it is doubtful as to whether its individuality is such as to justify its status as the sole representative of a separate genus. It seems preferable, therefore, to include it in the same genus as the coliform bacteria as *Bacterium friedländeri*.

Types. In spite of a number of attempts it has not been possible to make use of fermentations or other biochemical reactions in subdividing *Bact. friedländeri*. Through the work of Julianelle,¹⁴ however, it has been found that these bacteria may be divided into sharply defined immunological types. Analogous to the situation in the pneumococci, two types of antigen are present.

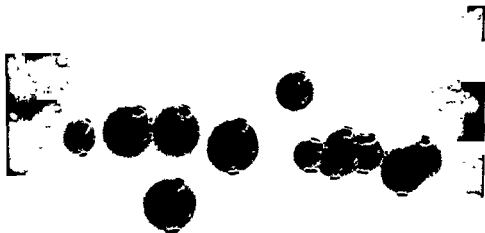


Fig. 68. Colonies of Friedländer's bacillus on blood agar. Note the large size and mucoid appearance. $\times 3$

The one, apparently nucleoprotein in nature, is species-specific, and the other, a polysaccharide present in the capsule, is type-specific. Julianelle has differentiated three serological types, Types A, B and C, to which the majority of strains belong, and a Group X for the remainder. Type B is immunologically similar to, although not identical with, Type 2 pneumococcus. The relative proportion of these types found in nature is indicated by Julianelle's study of 80 strains; 42 belonged to Type A, 12 to Type B, 7 to Type C and 19 to Group X. The Type A strains were largely from human sources and the Type B strains from lower animals. He has also reported that in Friedländer's bacillus pneumonias, Type A was found in 64 per cent, Type B in 14 per cent, Type C in 7 per cent and Group X in 15 per cent.

Pathogenicity. Microorganisms of this group are not infrequently found associated with various upper respiratory infections in man, though it is likely that in most instances they are secondary invaders. They are commonly present in the nasopharynx of persons suffering from chronic sinusitis or chronic lung infections such as bronchiectasis. Pneumonia due to Friedländer's bacillus is rare and makes up less than 1 per cent of all pneumonia, but the case fatality

¹⁴ Julianelle Jour. Exp. Med., 1926, 44 113, 693, 735, *ibid.*, 1930, 52 539, Ann. Int. Med., 1941, 51-190.

belonging to the same genus, is a frequent cause of the same type of infection, either invading the blood stream directly from the lung, or from some suppurative focus such as an empyema. *Staph. aureus* may produce a purely septicæmic infection following a localized infection; a staphylococcal "scarlet fever" when the toxæmia of acute or fulminating infection results in a generalized erythema (Stevens 1927, Simpson 1953); and acute lymphangitis. The generalization of a staphylococcal infection more commonly takes the form which is described in the following section. The occurrence of a transitory invasion of the blood stream by streptococci of the viridans type in cases of oral sepsis will be referred to in Chapter 68 (p. 1715). Attention may be drawn to the occurrence of septicæmia due to the non-sporing Gram-negative anaerobic bacilli of the *Fusiformis* group. This type is particularly liable to follow anginal infections, though it sometimes occurs after appendicitis or endometritis (see Chapter 79). Septicæmia due to anaerobic streptococci also occurs.

Infections by anaerobic bacteria are liable to be overlooked because diagnostic culture of the blood is seldom made anaerobically; and in the same way infections with bacteria that need an excess of carbon dioxide for growth (Chapter 3) will not be discovered unless a sample of blood is cultivated in air containing 5-10 per cent CO_2 (see Khairat 1910).

The great majority of bacteria which produce suppurative lesions may on occasion give rise to a secondary septicæmia; and in almost all fatal cases of this type such an invasion of the blood stream occurs during the terminal stages of the disease. Thus a septicæmia occurs in cases of acute peritonitis, in rare cases of acute gonococcal infection, and so on. In some meningococcal infections, the septicæmia appears to precede the appearance of purulent meningitis. Other bacteræmic organisms include the coliform bacilli, *Proteus* spp. and more rarely chromogenic organisms like *Ps. pyocyanea* and *Chr. prodigiosum*.

It is sometimes asserted that in septicæmia organisms are multiplying in the blood, whereas in bacteræmia they are derived from infected tissues. Such a distinction clearly cannot be drawn from the results of blood culture; it rests indeed on dubious clinical inference. The large and rapid increase in the number of circulating bacteria that sometimes takes place just before death suggests multiplication in the blood stream; but with this exception, the septicæmic phase of any bacterial infection is probably the expression of a rapid and continuous invasion of the blood stream from the tissues, either from a single focus which is active and extensive, or from the multiple foci which have been established earlier in the disease. In the frankly suppurative diseases, in which the primary or secondary foci are accessible to surgical interference, the results of adequate drainage testify to the efficiency of the clearing mechanism of the blood stream, if continuous re-invasion can be prevented.

Pyæmia.

This term is employed clinically to designate a type of general infection, the mechanism of which differs, to some extent, from that referred to above. In some bacterial infections, and particularly in the more severe forms of infection with *Staph. aureus*, such as acute osteomyelitis, metastatic abscesses may occur in such situations as the kidney, the brain, or the myocardium. In rare cases of appendicitis, or of other suppurative lesions in the abdominal cavity, multiple abscesses may occur in the liver, in direct relation to the portal vein—a condition

Moltke¹⁸ found that production of hydrogen sulfide and the decomposition of urea distinguish *Proteus* from all other gram-negative gelatin-liquefying bacilli. In a study of 194 strains he observed a definite division on the basis of maltose fermentation; 37 fermented maltose and 157 did not. All but one of the maltose-positive strains produced indol and none of the maltose-negative did. There were other correlated qualities. At the present time the bacteria comprising the genus *Proteus* are classified entirely on a biochemical basis. As noted above, the group is distinguished by its ability to hydrolyze urea; the liquefaction of gelatin is no longer regarded as a significant character. Four species are recognized, *Proteus vulgaris*, *Proteus mirabilis*, *Proteus rettgeri* and *Proteus morganii*; the last is known in the earlier literature as Morgan's bacillus (see below). These are distinguished from one another on the basis of the fermentation of mannitol, maltose and sucrose and the formation of indol.

Antigenic Structure. *Proteus* contains both H and O antigens (p 301) when motile, and the non-motile, non-swarming strains contain only O



Fig. 70. *Proteus vulgaris* colony on blood agar. Note the swarming exhibited as successive waves of growth (Dack).

antigen. The species of *Proteus* are immunologically heterogeneous but common antigens are frequently encountered and an immunological classification of these bacteria has not been practical. Certain *Proteus* strains are agglutinated by the serum of patients having typhus fever. These so-called X strains contain an antigen common to the typhus rickettsiae and the agglutination of these bacilli (the Weil-Felix reaction) is of diagnostic value in typhus fever (p. 820). In the *Proteus* strains the antigen is a part of the O antigen and its specificity is determined by an alkali-stable carbohydrate haptene which is also found in the *Rickettsia prowazekii*.¹⁹ The best known of the X strains is X-19. The X strains, it may be noted, frequently ferment maltose.

Pathogenicity. *Proteus*, both in mixed and pure cultures, has been found to be associated with a variety of pathological conditions. Infections of the eye and ear, pleuritis and peritonitis and suppurative abscesses in many parts of the body are among the many instances in which the pathogenic power of this bacterium in pure culture can hardly be doubted. As a producer of cystitis it probably ranks next to *Bacterium coli*. Besides its independent

¹⁸ Moltke. Contribution to the Characterization and Systematic Classification of *Bact. proteus vulgaris* (Hauser). Copenhagen. 1927.

¹⁹ Cf. White. Brit Jour. Exp. Path., 1933, 14, 145, Castaneda. Jour. Exp. Med., 1934, 60:119. *ibid.*, 1935, 62, 289.

of soft nodes of granulation tissue situated in a network of fibrous strands. Embedded in the granulation tissue are small yellowish-white granules about the size of a grain of sand. These consist of grape or mulberry-like clusters of Gram-positive micrococci surrounded by a zoogloal substance. The granules are sometimes referred to as Bollinger's granules, and the organism has been called *M. ascoformans*, though there is little doubt that it is in fact *Staph. aureus*. A similar picture is seen in mammary botriomycosis of swine. In cattle, however, the colonies are surrounded by definite clubs instead of by an undifferentiated mass, and thus bear a close resemblance to true actinomycosis. In all three animals the disease may be reproduced by experimental inoculation of *Staph. aureus* under suitable conditions. Similar staphylococcal lesions have been observed on at least 10 occasions in man (Drake *et al* 1945).

Allied to botriomycosis is an enzoötic staphylococcal infection of lambs, sometimes referred to as *tick pyæmia*. It is common in Northumberland, Scotland and Ireland. Infected lambs may die from septicæmia a few days after birth; those that escape develop chronic abscesses of the joints and liver; the meninges may also be invaded. The disease is caused by *Staph. aureus*. No satisfactory specific means of prevention is yet known (see McDiarmid 1946, Foggie 1948).

Streptococcal Infections of the Skin and Subcutaneous Tissues.

Erysipelas, an acute spreading infection of the true dermis, has been considered in Chapters 53 and 66.

Impetigo contagiosa, a contagious and sometimes epidemic infection of the superficial layers of the skin characterized by early vesiculation and subsequently by weeping, yellowish-brown, scabbed lesions, is usually thought to be streptococcal in origin. It is in fact associated with Group A streptococci, *Staph. aureus*, or with both (Cruickshank 1941). In a total of 180 patients, Bigger and Hodgson (1943, 1944) found the staphylococcus in 98.8 per cent. and the streptococcus in 36.7 per cent. The streptococcus was seldom found alone and generally appeared late in the disease, suggesting a secondary infection. Cruickshank (1953) distinguishes the streptococcal variety, in which the organism is found in the blister fluid in the early stages, and which occurs most commonly in children aged 5-9, from the bullous staphylococcal variety discussed on p. 1685. (See also Friedberg 1945.)

Cellulitis is an acute spreading infection of the subcutaneous tissue, characterized by the formation of a sero-purulent, often blood-stained exudate, with no evidence of localization. Rapid lymphatic spread, and septicæmic generalization, are far more frequent than from localized staphylococcal infections. In very acute infections, such as those which may follow post-mortem wounds, the local manifestations may be minimal, the spread being so rapid that an acutely fatal septicæmia dominates the clinical picture from the first. In these cases the streptococcus concerned is almost always *Str. pyogenes*.

It may be noted that a comparison of the modal types of staphylococcal and streptococcal infection affords an excellent example of the importance of differences in the biological characters of bacteria, in determining the type of lesion which they produce when they gain access to the tissues.

Mastitis in Cattle.—This important disease can only be touched on briefly here. Those seeking further information are referred to the monograph by Steck (1930) and to the series of papers by Minnett and his colleagues in this country (Minnett, Stableforth, and Edwards 1929, 1930, 1932, 1933, Minnett and Stableforth 1931,

pathogenicity, it is found so commonly associated with other microorganisms in purulent war wounds and similar processes that its activity as an accomplice is probably second only to that of the cocci.

In certain affections of the digestive tract *Proteus* has been frequently held to be the responsible agent. In diarrheic stools, especially those of infants, it has often been found in large numbers, and is regarded by many as a cause of infant diarrhea.²⁰ The real relation of *Proteus* to intestinal infection is, however, still obscure.

Certain food-poisoning epidemics have been ascribed to *Proteus*. These cases have been collected and subjected to a critical analysis by Bengtson,²¹ who concludes that a definite proof of the causal relationship between the bacterium isolated and the illness is lacking in all instances. Further evidence is needed to establish the role of *Proteus* in food poisoning.

Animal inoculation shows that a great range of virulence exists among the *Proteus* cultures that have been tested. Freshly isolated strains from pathological sources may produce definite lesions, including abscesses, enlargement of the spleen and a diarrheic condition. One strain from a case of peritonitis has been reported which killed mice in amounts of 0.005 ml. of broth culture. The etiologic agent of "red-leg" disease of frogs, "ulcer disease" of brook trout, and "red sore" of pike has been found to be a bacillus closely related to the *Proteus* group and it has been classified as *Proteus hydrophilus*.²² The cell substance of these bacteria is toxic on parenteral injection and, as in the case of the enteric forms, appears to be a glucolipid. No difference in toxicity is apparent between strains of pathogenic and non-pathogenic origin.

Morgan's Bacillus. This bacillus was isolated by Morgan²³ from the stools of infants suffering from summer diarrhea. Although it was first designated as "Morgan's No. 1," the other varieties of "Morgan's bacilli" have long since faded out of general interest and recognition, so that the name Morgan bacillus is usually applied to "Morgan's No. 1." In its cultural characteristics Morgan's bacillus resembles the coliform bacteria. Although it does not liquefy gelatin, it is closely related to the *Proteus* group, and it is therefore classified as *Pr. morgani*.

This bacillus appears to have played a part in a number of outbreaks of summer diarrhea of infants and has been isolated from paratyphoid like fevers. It has been found to give rise to spontaneous epidemics of enteritis in mice. Injected intraperitoneally into mice, Morgan's bacillus produces a rapidly fatal infection.

²⁰ Cf. Neeter and Farrar: Amer. Jour. Digest. Dis., 1943, 10 344.

²¹ Bengtson: Jour. Inf. Dis., 1919, 24 429.

²² Kulp and Borden: Jour. Bact., 1942, 44 673, Reed and Toner: Canadian Jour. Res., Sec. C, 1942, 20 161.

²³ Morgan: Brit. Med. Jour., 1906, 1908.

the year. It is far commoner in dry cows than in cows in milk, and usually develops shortly before calving. One or more quarters of the udder are affected. Constitutional symptoms, sometimes accompanied by swelling of the hock joints, are not uncommon. The disease is often chronic, and abscesses in the udder tissue may break through the skin. Microscopical examination of the udder secretion reveals enormous numbers of Gram-positive diphtheroid bacilli, which, when cultivated on coagulated blood serum, give the typical pitted appearance due to liquefaction. It should be noted that *C. pyogenes*, besides being associated with summer mastitis, is responsible for a variety of other suppurative infections in cattle.

STAPHYLOCOCCAL MASTITIS.—This form usually occurs a few days after calving. One or more quarters of the udder are affected. The disease is sometimes acute, accompanied by gangrene, and may prove fatal. More often it is of the chronic catarrhal type (see Minett 1937). The discharge from the affected quarters contains large numbers of hæmolytic coagulase-positive staphylococci—*Staph. aureus*. Occasionally a chronic granulomatous disease develops, usually referred to as actinomycosis, but more properly termed botriomycosis (see p. 1463).

Diagnosis of Mastitis.—In its more severe forms mastitis can be detected by clinical means alone, but the chronic streptococcal types of infection usually call for laboratory assistance. Individual quarter samples of milk are preferred for examination, the fore-milk being the most suitable. In well-developed cases of the streptococcal disease flakes in the fore-milk are visible against a dark background; the reaction of the milk is more alkaline than normal; on centrifugation of the milk the cellular sediment is found to be increased and to exceed 1 part per 1,000; by the Breed smear method the cells usually number more than one million per ml.; microscopical examination of the centrifugal deposit reveals the presence of fairly long-

TABLE 117

DIFFERENTIAL REACTIONS OF MASTITIS STREPTOCOCCI (MODIFIED FROM SLAVIN 1949)

	Hæmo- lysis on Blood Agar	Sor- bitol	Tre- halose	Raffi- nose.	Inulin.	Salicin	Æsculin	Sodium Hippu- rate.	NB, from Argi- line
<i>Str. pyogenes</i>	+	—	+	—	—	+	?	—	+
<i>Str. agalactiæ</i>	±	—	+	—	—	+	—	+	+
<i>Str. dysgalactiæ</i>	—	+	+	—	—	+	—	—	+
<i>Str. zoëpidemicus</i>	+	+	—	—	—	+	?	—	+
<i>Str. uberis</i>	+	+	+	—	+	+	+	+	+

chained streptococci; and cultivation of the centrifuged deposit shows the presence of streptococci which, on further examination, are found to belong to one of the mastitis groups (see Table 117). The most delicate test consists in the cultivation of the gravity cream or the centrifuged deposit, preferably in deep blood agar containing æsculin and crystal violet (Edwards 1933). For herd tests, composite milk samples from the udders of each cow may be incubated in the presence of sodium azide and bromocresol purple as a preliminary means of detecting milks containing *Str. agalactiæ* (Edwards 1938). (See also Plastringe *et al.* 1946, Cunningham *et al.* 1946, Chu 1949, McEwen and Cooper 1947, Slavin 1948.)

Prevention and Treatment of Mastitis.—The early observations of Minett and his colleagues led these workers to suggest a modified eradication plan for dealing

THE ENTERIC BACILLI: THE SALMONELLA GROUP¹

The first member of this large group was described in 1888 by Gartner, who isolated it from diseased beef responsible for an outbreak of gastro-enteritis, and named it *Bacillus enteritidis*. Similar bacteria have been found frequently in foods epidemiologically implicated in outbreaks of food poisoning, others are responsible for outbreaks of disease in rats, mice and other rodents, others are found in the paratyphoid fevers in man, and still others are responsible for certain poultry diseases.¹ These bacteria are parasitic and, although widely distributed in nature, do not maintain a saprophytic existence.

Morphology and Staining. These bacilli are gram-negative rods closely resembling and indistinguishable from the coliform bacteria. They stain readily with the usual dyes such as methylene blue and carbol fuchsin. No particular arrangement of the cells is apparent on microscopic examination. All species except *S. pullorum* and *S. gallinarum* are actively motile by means of peritrichous flagella. No capsules are apparent and spores are not formed.

Physiology. The bacteria of this group have simple nutritional requirements, growing readily on the usual nutrient media. In synthetic media an ammonium salt and glucose, pyruvate, lactate, etc. are adequate sources of nitrogen and carbon, and the great majority of strains do not require bacterial vitamins or amino acids.² The optimum temperature is 37° C. but growth occurs at a reasonable rate at room temperature. They are facultative anaerobes, growing equally well under either aerobic or anaerobic conditions, and some species develop relatively strong reducing intensities.

The group is characterized biochemically by failure to ferment lactose or salicin, and inability to liquefy gelatin or produce indol. There are a very few exceptions to the two last, *S. eastbourne* and some strains of *S. enteritidis* and *S. panama* produce indol, and *S. dar-es-salaam* liquefies gelatin. The evolution of gas commonly accompanies the fermentation of sugars though anaerogenic strains of *S. enteritidis*, *S. typhi-murium* and *paratyphi C* have been reported.

In the past considerable significance has been attached to the differential biochemical reactions of these bacilli and they have been separated on this basis. In recent years, however, the antigenic structure of these bacteria has been emphasized and antigenic analysis has been carried further with this group than with any other bacteria. The differential value of sugar fermentations and other biochemical reactions remains of primary importance nevertheless. The biochemical reactions of some of the more frequently encountered *Salmonella* species are given in the accompanying table (p 441).

¹ For a comprehensive discussion of these bacteria see Kauffmann: *The Bacteriology of the Salmonella Group*. Munksgaard, Copenhagen 1941.

² Lederberg. *Arch Biochem.*, 1947, 13:287

Jamieson and Stuart (1950) described a small outbreak of infective endocarditis in young lambs unassociated with joint lesions and apparently caused by *Str. focalis*.

Group C streptococci are commonly found in infections of the horse. Bazeley and Battle (1940) described five sub-types of equine Group C streptococci, all of which were associated with respiratory catarrhs, wound infections and suppurative lesions in various parts of the body. Their Type 1 appeared to be the causal organism of *strangles*, a suppurative lesion of the upper respiratory tract, characterized by abscesses in the throat and submaxillary region. In India, however, Rajagopalan and Israil (1945) found Type 4 most frequently. Bazeley (1940) was able to immunize mice against infections with Type 1, using vaccines of heat-killed capsulated streptococci.

Conjunctivitis.—One of the commonest causes of acute conjunctivitis is the Koch-Weeks bacillus or the closely related *H. influenza* (see Chapter 33; Davis and Pittman 1950). This disease appears to be widespread, occurs in epidemic form and shows a definite seasonal prevalence in different countries. It is common in Egypt, where the bacillus was first isolated by Koch. Another, less common, cause of conjunctivitis is the pneumococcus. This type of infection, which is more severe than that due to the Koch-Weeks bacillus, is irregular in distribution, is confined chiefly to children, and may occur in localized epidemics. It is more common in Eastern than in Western countries. Subacute, or angular, conjunctivitis is due to the Morax-Axenfeld bacillus (see Chapter 34). Gonococcal conjunctivitis—ophthalmia neonatorum—is described in Chapter 65. *Staph. aureus* is associated with a mild inflammatory disease in infants known as “sticky eye” (see Taylor and Calman 1948). (For a general account of the bacteriology of conjunctivitis see Axenfeld 1928.)

Suppurative Lesions in Bones and Joints.—Acute osteomyelitis, occurring independently of a compound fracture, is almost always due to infection with *Staph. aureus*. It occurs mainly in children, in whom it not infrequently develops after a simple blow, or similar trauma. The infection is blood-borne. Acute suppurative periostitis is rare. When it occurs, it is usually a streptococcal infection. A subacute, or chronic, periostitis occasionally occurs as a sequel of typhoid or paratyphoid fever, and is caused by the bacillus responsible for the primary infection. Such abscesses occur most commonly over the ribs, or the cranium.

A metastatic purulent arthritis may occur as a sequel to infection with any pyogenic bacterium; though the pneumococcus is the organism most frequently isolated in such cases. Other bacteria are rarely isolated from true suppurative arthritis in man, except in those cases which follow wounds entering the joint. In animals other bacteria are often responsible for this condition, as, for instance, *Corynebacterium pyogenes* and *Erysipelothrix rhusiopathiae*.

Suppurative Lesions of the Alimentary Tract.

Appendicitis.—The bacteriology of acute appendicitis, and of the associated appendix abscesses, is very confused. It is possible that the primary lesion occurs in the wall of the appendix, and that it is due to a blood-borne infection with a streptococcus of the viridans type (see Rosenow 1915). The complex bacterial flora of this part of the intestine ensures a rapid secondary invasion of the damaged tissue with a host of different bacteria, among which *Bact. coli* is prominent; and the bacteriology of the lesion found at operation is correspondingly complex.

Veillon and Zuber (1898), using a combination of aerobic and anaerobic methods,

Toxins. No soluble toxin is formed by the bacteria of the *Salmonella* group, but, as is the case of many other bacteria, their cell substance is toxic upon parenteral inoculation. This toxic quality of two species, *S. enteritidis* and *S. aertrycke*, has been investigated and found to be a property of contained glucolipids (p. 205 and p. 284) which have immunological individuality (somatic antigens) and hence are, in a sense, specific. Pharmacologically, however, they are not specific, and their activity does not differ to a marked degree from that of the cell substance of other bacteria.³

The Immunological Differentiation of *Salmonella* Bacilli. The techniques of antigenic analysis have been developed through the efforts of White⁴ in the study of the *Salmonella* group, and the antigenic composition of bacteria of this group is, perhaps, better known than that of any other

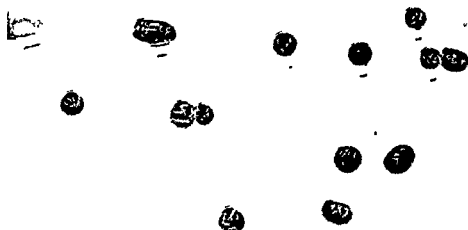


Fig 71. Colonies of *Salmonella typhi-murium* (*aertrycke*) on nutrient agar. Twenty-four hour culture $\times 3$.

bacteria. Many members of the group are immunologically complex, whether because they are innately so or because they are better known in this respect than most other bacteria is difficult to say.

Flagellar and Somatic Antigens. Two types of antigens are present in the bacteria of the *Salmonella* group, one associated with the cell substance and the other with the flagella. Early noted by Smith and Reagh,⁵ the first was termed *somatic antigen* and the second *flagellar antigen*. These types were later observed by Weil and Felix,⁶ who designated them *O* and *H* antigens respectively (p. 301). The *O* antigens are arbitrarily designated by Roman numerals. Two species of *Salmonella*, *S. paratyphi* C and *S. ballerup*, also contain *V_i* antigen (p. 451); this antigen is heat-stable and in this and certain other respects appears to be closely related to the *O* antigens.

These two types of antigen, each of which may be, and frequently is, represented in a single bacterial strain by more than one component, differ from

³ See Cameron, Delafield and Wilson

⁴ White. Med. Res. Council Spec. Re

⁵ Smith and Reagh. Jour. Med. Res

⁶ Weil and Felix. Ztschr. f. Immuni

et., 1940, 51, 223.

93, 1926.

79 24.

in 43 cultures from the lymph node. He concluded that bile, by its inhibitory action in culture, prevents the detection of the streptococcus, which is the cause of the primary infection. These observations clearly suggest that *Bact. coli* may, in many cases, play the part of an important secondary invader, favoured by the selective action of the bile, rather than that of a primary agent. León and Campuzano (1946) found bacteria in 13.4 per cent. of biliary samples from persons without cholecystitis, and in 84.4 per cent. from those with cholecystitis. The percentage frequency of the most common bacteria were: enterococci, 43.7; coliform bacilli, 30.0; and *Staph. aureus* 27.1.

Peritonitis.—Some cases of peritonitis are primary, for example, certain cases in children due to infection with the pneumococcus; but the great majority of cases are secondary to some primary intra-abdominal lesion, such as appendicitis, perforated gastric or duodenal ulcer, or some type of intestinal obstruction. The species of bacteria concerned in the primary invasion of the peritoneum depends in part on the nature of the primary lesion, in part on the flora of the section of the intestinal tract involved. In intestinal obstruction the bacteria from within the intestine tend to make their way through the intestinal wall, and to produce a mixed infection. In such cases, and in the later stages of any infection of the general peritoneal sac, other than those which are acutely fatal, the bacteriological picture is usually dominated by the colon bacillus.

Fatal post-operative peritonitis is often due to infection with a hæmolytic streptococcus, and a similar infection may occur as part of a septicæmic infection. Pelvic peritonitis, following salpingitis, is usually due to the gonococcus. Other bacteria which may be found in the peritoneal exudate include *Staph. albus*, bacilli of the *Proteus* group, and *Ps. pyocyanea* (see Dudgeon and Sargent 1905). In those cases in which free transit has occurred from the interior of some part of the lower intestine, anaerobes of the *Fusiformis* and *Clostridium* groups may be present. Cazzamali and Miglierina (1933), who examined 81 cases of acute peritonitis, found that aerobic organisms accounted for about two-thirds and anaerobic for about one-third of the total number isolated. In point of time the aerobic organisms preceded the anaerobic. *Bact. coli* seemed to be able to diffuse more rapidly through the peritoneal cavity than any other organism, though streptococci persisted for the longest time. In cases of perforated gastric or duodenal ulcer no organisms could be detected for 6, or even 12 hours, but after this time the peritoneal cavity was always infected, the commonest organism being a non-hæmolytic streptococcus. From a prognostic point of view, excluding infections with *Str. pyogenes*, the fewer organisms and the fewer species there are present, the more favourable is the outlook (see also Meleney *et al.* 1931).

Suppurative Lesions of the Respiratory Tract.

Suppurative lesions of the accessory sinuses of the nose usually arise by direct extension from the nares or nasopharynx, and the bacteria most frequently responsible are the potentially pathogenic constituents of the nasopharyngeal flora—*Str. pneumoniae*, *Str. pyogenes*, and *H. influenzae*. Another organism frequently isolated from cases of suppurative sinusitis is *Staph. aureus*. Acute infection of the nasal sinuses with the meningococcus has been described, and is believed by some observers to play a part in the genesis of cerebrospinal meningitis (see Chapter 64).

Under this heading, we may, for convenience, include otitis media. The great majority of cases in man are caused by *Str. pyogenes*, *Str. pneumoniae*, or *Staph.*

THE SALMONELLA TYPES (SPECIES)

Immunologic Types		Antigenic Formulae*		
Group	Species	Somatic Antigens	Flagellar Antigens	
			Phase 1	Phase 2
A	<i>S. paratyphi A</i>	(I), II, XII	a	
B	<i>S. paratyphi B</i>	(I), IV, (V)	b	(1, 2)
	<i>S. abony</i>	(I), IV, V	b	e, n, x
	<i>S. typhi-murium</i>	(I), IV, (V)	(i)	1, 2, 3
	<i>S. stanley</i>	IV, V	d	1, 2
	<i>S. heidelberg</i>	IV, V	r	1, 2, 3
	<i>S. chester</i>	IV, (V)	e, h	e, n, x
	<i>S. san diego</i>	IV, (V)	e, h	e, n, z ₁₃
	<i>S. salinatis</i>	IV	d, e, h	d, e, n, z ₁₃
	<i>S. saint paul</i>	I, IV, V	e, h	1, 2, 3
	<i>S. zagreb</i>	IV, V	e, h	1, 2
	<i>S. reading</i>	IV	e, h	1, 5
	<i>S. kapostar</i>	IV, V	e, (h)	1, 5
	<i>S. koeln</i>	IV, V	y	1, 2
	<i>S. kaapstad</i>	IV	e, h	1, 7
	<i>S. derby</i>	(I), IV	i, g	
	<i>S. essen</i>	IV	g, m	
	<i>S. budapest</i>	I, IV	g, t	
	<i>S. californica</i>	IV	g, m, t	
	<i>S. brandenburg</i>	IV	l, v	e, n, z ₁₃
	<i>S. bispebjerg</i>	I, IV	a	e, n, x
	<i>S. abortus equi</i>	IV	a	e, n, x
	<i>S. arechavaleta</i>	IV, (V)	a	1, 7
	<i>S. abortus ovis</i>	IV	c	1, 6
	<i>S. allendorf</i>	IV	c	1, 7
	<i>S. texas</i>	IV, V	k	e, n, z ₁₃
	<i>S. stanleyville</i>	IV, V	z ₆ , z ₂₃	
	<i>S. oahu</i>	IV, V	l, v	1, 2, 3
	<i>S. abortus bovis</i>	(I), IV, XXVII	b	e, n, x
	<i>S. bredeney</i>	I, IV, (XXVII)	l, v	1, 7
	<i>S. schleissheim</i>	IV, XXVII	b, z ₁₃	
	<i>S. schwarzengrund</i>	I, IV, XXVII	d	1, 7
C-1	<i>S. paratyphi C</i>	VI, VII, (Vi)	c	1, 5
	<i>S. cholerae-suis</i>	VI, VII	(c)	1, 5
	<i>S. typhi-suis</i>	VI, VII	(c)	1, 5
	<i>S. thompson</i>	VI, VII	(k)	1, 5
	<i>S. montevideo</i>	VI, VII	g, m, s	
	<i>S. oranienburg</i>	VI, VII	m, t	
	<i>S. virchow</i>	VI, VII	r	1, 2, 3
	<i>S. oslo</i>	VI, VII	a	e, n, x
	<i>S. amersfoort</i>	VI, VII	d	e, n, x
	<i>S. braenderup</i>	VI, VII	c, h	e, n, z ₁₃
	<i>S. potsdam</i>	VI, VII	l, v	e, n, z ₁₃
	<i>S. bareilly</i>	VI, VII	y	1, 5
	<i>S. hartford</i>	VI, VII	y	e, n, x
	<i>S. mikawashima</i>	VI, VII	y	e, n, z ₁₃
	<i>S. tennessee</i>	VI, VII	z ₁₉	
	<i>S. infantis</i>	VI, VII	r	1, 5
	<i>S. concord</i>	VI, VII	l, v	1, 2, 3

* Naturally occurring diagnostic formulae; experimental phase alterations not taken into account.

Legend: () — antigen may be absent; [] — incomplete antigen

associated with a recto-vesical fistula. Cystitis is a not uncommon complication of gonorrhoea, and is then due to the gonococcus, alone or in association with other organisms. Tuberculous cystitis or pyelonephritis, which is itself associated with pyuria, is frequently complicated during its later stages by a secondary infection with *Bact. coli*, staphylococci, or other organisms.

Infections of the bladder and of the pelvis of the kidney provide another illustration of the importance of local mechanical factors in promoting bacterial infection. Renal or vesical calculi, enlarged prostate, urethral stricture, vesical papilloma or carcinoma, are all conditions which, sooner or later, are associated with an infective cystitis. *Staph. aureus* is the organism most commonly found in urinary calculi, both in the stone itself, and in the adjacent tissues (Helfstrom 1924, Winsbury-White 1935).

Pyelonephritis is sometimes observed in cattle and is characterized by a membranous necrotic inflammation, which attacks principally the papillas and leads to progressive destruction of the kidney; there is an accompanying inflammation of the capsule, and often considerable fibrosis of the pelvis of the kidney, the ureter, and the bladder. The disease is produced by the Gram-positive diphtheroid bacillus *C. renale*, alone or sometimes mixed with streptococci, coliform bacilli, *C. pyogenes* or other organisms (Glage 1928, Jones and Little 1930). Though it commonly attacks adult and particularly female cattle, the bacillus may be acquired in early life, and is carried in the vagina (Weitz 1917) or urinary tract. It appears to have a predilection for the medulla of the kidney, but it is not clear whether infection reaches the tissue *via* the blood stream or as an ascending infection from the urinary tract (Lovell 1951). The disease should be regarded as contagious (Morse 1950). Prolonged treatment with penicillin appears to offer the best hope of a cure (Lovell and Cotchin 1947, Lovell 1951).

Infection of Wounds

Bacterial infection is a common sequel to wounding. All wounds, from trivial cuts to the extensive areas of destruction that follow burning, are liable to infection, though in general the larger and deeper the wound and the more damaged and "devitalized" the wounded tissues, the greater is the susceptibility to infection. The risk of infection to a clean operation wound, closed so as to heal by first intention, is relatively small; and that of an extensive compound fracture, with crushing or laceration of the tissues, is high. Few organisms are introduced into a clean operation wound, and after operation the wound can be adequately protected against subsequent infection. The same is true of the "physiological" wounds in the genital tract of the post-parturient woman; sepsis does not develop if precautions are taken against the introduction of bacteria during and after labour. The accidental wound, whether inflicted in civil life or in war, differs in that the tissues are usually more damaged, and in the greater likelihood that the wound will be contaminated by bacteria present in the environment of the wound at the time of infliction. The bacteria include those from the skin and clothes of the wounded person, and those from the soil, dust, dirt and other debris that fall on or are driven by force into the wound. Infection in these wounds is much commoner and has been studied more extensively than has infection of clean wounds. There is, however, no essential distinction between the two kinds, and our conclusions will apply with varying force to infection of all open lesions of the body. It will be convenient to make an arbitrary distinction between the bacteria

THE *SALMONELLA* TYPES (SPECIES)—Continued

Immunologic Types		Antigenic Formulae*		
		Somatic Antigens	Flagellar Antigens	
Group	Species		Phase 1	Phase 2
C-1 (Cont.)	<i>S. georgia</i>	VI, VII	b	e, n, z ₁₃
	<i>S. papiana</i>	VI, VI ₁ , VII	r	e, n, z ₁₃
	<i>S. richmond</i>	VI, VII	y	1, 2, 3
	<i>S. cardiff</i>	VI, VII	k	1, 10
	<i>S. daytona</i>	VI, VII	k	1, 6
	<i>S. mission</i>	VI, VII	d	1, 5
	<i>S. singapore</i>	VI, VII	k	e, n, x
C-2	<i>S. new port</i>	VI, VIII	(e, h)	1, 2, 3
	<i>S. kottbus</i>	VI, VIII	e, h	1, 5
	<i>S. muenchen</i>	VI, VIII	d	1, 2
	<i>S. mexicana</i>	VI, VIII	d	1, 2, 4
	<i>S. pueris</i>	VI, VIII	e, h	1, 2
	<i>S. oregon</i>	VI, VIII	d	1, 2, 3
	<i>S. manhattan</i>	VI, VIII	d	1, 5
	<i>S. litchfield</i>	VI, VIII	l, v	1, 2, 3
	<i>S. morbihcans boris</i>	VI, VIII	r	1, 5
	<i>S. narashino</i>	VI, VIII	a	e, n, x
	<i>S. bonariensis</i>	VI, VIII	i	e, n, x
	<i>S. glostrup</i>	VI, VIII	z ₁₀	e, n, z ₁₃
	<i>S. duesseldorf</i>	VI, VIII	z ₄ , z ₁₁	
	<i>S. tallahassee</i>	VI, VIII	z ₄ , z ₂₂	
	<i>S. gatuni</i>	VI, VIII	b	e, n, x
	<i>S. hidalgó</i>	VI, VIII	r	e, n, z ₁₃
	<i>S. izo-jima</i>	VI, VIII		1, 5
	<i>S. amherst</i> (VIII)	(VIII)	l, v	1, 6
	<i>S. virginia</i> (VIII)	(VIII)	d	
D	<i>S. typhi</i>	IX, (X)	d	
	<i>S. enteritidis</i>	(I), IX	g, m	
	<i>S. dublin</i>	I, IX	g, p	
	<i>S. rostock</i>	I, IX	g, p, u	
	<i>S. moscow</i>	IX	g, q	
	<i>S. lleedam</i>	IX	g, m, q	
	<i>S. berla</i>	IX	f, g, t	
	<i>S. pensacola</i>	IX	g, m, t	
	<i>S. clausborne</i>	I, IX	k	1, 5
	<i>S. serdai</i>	(I), IX	a	1, 5
	<i>S. loma linda</i>	IX	a	e, n, x
	<i>S. durban</i>	IX	a	e, n, z ₁₃
	<i>S. anatum</i>	I, IX	b	1, 2
	<i>S. eastbourne</i>	(I), IX	e, h	1, 5
	<i>S. panama</i>	I, IX	l, v	1, 5
	<i>S. dar-es salaam</i>	I, IX	l, w	e, n
	<i>S. goettingen</i>	IX	l, v	e, n, z ₁₃
	<i>S. jamaica</i>	(I), IX	l, z ₁₁	1, 5
	<i>S. gallinarum</i>	IX		
	<i>S. pullorum</i>	IX		
	<i>S. canastot</i>	IX		
	<i>S. italica</i>	IX	z ₁₁	1, 3, 5
	<i>S. napoli</i>	(I), IX	l, v	1, 11
	<i>S. new york</i>	IX	l, z ₁₁	e, n, x
	<i>S. new mass</i>	IX	l, v	1, 5
			a	1, 5

infecting and carrier strains through a community, led to the elucidation of staphylococcal epidemics other than those among wounds. Newly-born infants are free from *Staph. aureus*, but, in hospitals at least, their noses are colonized within a few days, and up to 96 per cent. may be carriers by the second week of life: the carrier rate is lower in infants at home, and it is only in the 6-15 year age group that it approaches that in adults (Cunliffe 1949; see also Rountree and Barbour 1950, Wallmark and Laurell 1952, Laurell and Wallmark 1953, Ludlam 1953). The higher carrier rate in hospital holds also for adults; the frequency is higher in the wards than in out-patient clinics, or in the outside world—evidence of greater staphylococcal hazard, which is strengthened by the observation that some incomers to wards acquire types of staphylococci carried by the hospital population (Miles *et al.* 1944, Barber *et al.* 1949, Rountree and Barbour 1951). In some surveys, no particular type is associated with carriage or infection (Williams 1946, Wallmark and Laurell 1952). However, Barber and her colleagues (1949), and subsequent workers, using penicillin-resistance as well as the phage type as a means of identification, found that one or a few phage types predominated among hospital populations infected with resistant cocci. There is, however, little to suggest that these predominating types have a peculiar infectivity for man.

It is evident from investigations of maternal mastitis, and of pemphigus and conjunctivitis in the newly born infant (Elhott *et al.* 1941, Allison and Hobbs 1947, Barber *et al.* 1949, Parker and Kennedy 1949, Denton *et al.* 1950, Oeding 1952), and of sycosis barbae (Hobbs *et al.* 1947), that the method of spread varies: it may be directly from infant to infant, or *via* mother and nurses; nasal colonization and the staphylococcal infection may increase concurrently or separately; and the nose may be infected from the lesion or the lesion from the nose. Attempts to check such outbreaks must obviously be designed to meet all the possibilities of spread between these various sites. Self infection, as well as cross-infection, is probably common. A relation between intractable furunculosis and persistent nasal carriage was suggested by Dolman in 1935; and Valentine and Hall-Smith (1952) record cases of furunculosis and sycosis barbae where not only was the same type of coccus present in the nose and the infected lesions, but successful treatment included measures to prevent further self-infection from the nose.

Epidemic outbreaks are associated with hospitals probably because of the ease with which the environment becomes heavily charged with *Staph. aureus*; but they are not confined to hospitals. In a family of 7 children, Harrison (1948) found the same phage type of coccus in two with acute suppurative arthritis, one with acute osteomyelitis and in the nasal mucosa of two others.

Treatment of Pyogenic Infections

Surgical.—Although the discussion of surgical methods of treatment takes us beyond our proper ground, it is permissible to point out that general bacteriological principles indicate the paramount importance of judicious surgical interference in those acute suppurative infections which are accessible to such measures. The removal, or adequate drainage, of a localized focus of infection will usually enable the defence mechanisms of the body to deal satisfactorily with the remaining bacteria. We have emphasized above the importance of mechanical factors in many cases of acute or chronic pyogenic infections; and the rectification of these abnormalities by surgical means frequently results in a bacteriological cure. Among many substances, enzymic and otherwise, that have been used to clear away necrotic, viscous or purulent material from infected cavities, we may note the modern use of two enzymes from *Str. pyogenes* itself—a fibrinolysin and a deoxy-

THE SALMONELLA TYPES (SPECIES)—Continued

Immunologic Types		Antigenic Formulae*		
Group	Species	Somatic Antigens	Flagellar Antigens	
			Phase 1	Phase 2
E-1	<i>S. london</i> (L II)	III, X, XXVI	l, v	1, 6
	<i>S. give</i>	III, X, XXVI	l, v	1, 7
	<i>S. uganda</i>	III, X, XXVI	l, z ₁₃	1, 5
	<i>S. anatum</i>	III, X, XXVI	e, h	1, 6
	<i>S. muenster</i>	III, X, XXVI	e, h	1, 5
	<i>S. nyborg</i>	[III, X, XXVI]	e, h	1, 7
	<i>S. vejle</i>	III, X, XXVI	e, h	1, 2, 3
	<i>S. macleodidis</i> ✓	[III, X, XXVI]	e, h	1, w
	<i>S. shangani</i>	III, X, XXVI	d	1, 5
	<i>S. canzibar</i>	[III, X, XXVI]	k	1, 5
	<i>S. amager</i>	III, X, XXVI	y	1, 2, 3
	<i>S. lexington</i>	[III, X, XXVI]	z ₁₀	1, 5
	<i>S. uellevreden</i>	III, X, XXVI	r	z ₆
	<i>S. orion</i>	III, X, XXVI	y	1, 5
	<i>S. butantan</i>	III, X, XXVI	b	1, 5
	<i>S. saipan</i>	III, X, XXVI	z ₄	1, 6
E-2	<i>S. newington</i>	III, XV	e, h	1, 6
	<i>S. scotlandia</i>	III, XV	e, h	1, 7
	<i>S. new brunswick</i>	III, XV	l, v	1, 7
	<i>S. illinois</i>	[III], [XV], XXXIV	z ₁₀	1, 5
	<i>S. cambridge</i>	III, XV	e, h	1, n
E-3	<i>S. senftenberg</i>	I, III, XIX	g, s, t	
	<i>S. nitroese</i>	I, III, XIX	d	z ₆
	<i>S. simsbury</i>	I, III, XIX	z ₁₇	
	<i>S. taksony</i>	I, III, XIX	i	z ₆
F	<i>S. hebes</i>	VI, XIV, XXIV	d	1, 5
	<i>S. carrau</i>	VI, XIV, XXIV	y	1, 7
	<i>S. onderstepoort</i>	[I], VI, XIV, XXV	e, [h]	1, 5
	<i>S. florida</i>	[I], VI, XIV, XXV	d	1, 7
	<i>S. madelia</i>	[I], VI, XIV, XXV	y	1, 7
	<i>S. suntschall</i>	(I), VI, XIV, XXV	z	e, n, x
	<i>S. kentucky</i>	[VIII], XX	i	z ₆
	<i>S. aberdeen</i>	XI	i	1, 2, 3
	<i>S. rubislaw</i>	XI	r	e, n, x
	<i>S. pretoria</i>	XI	k	1, 2, 3
	<i>S. solt</i>	XI	y	1, 5
	<i>S. luciana</i>	XI	a	e, n, z ₁₃
	<i>S. venezia</i>	XI	i	e, n, x
	<i>S. senegal</i>	XI	r	1, 5
	<i>S. marseille</i>	XI	a	1, 5
	<i>S. chandans</i>	XI	d	e, n, x
	<i>S. grumpensis</i>	XIII, XXII	d	1, 7
	<i>S. poona</i>	XIII, XXII	z	1, 6
	<i>S. borbeck</i>	XIII, XXII	l, v	1, 6
	<i>S. mississippi</i>	I, XIII, XXIII	b	1, 5
	<i>S. wchuta</i>	I, XIII, XXIII	d	
	<i>S. havana</i>	I, XIII, XXIII	f, g	z
	<i>S. worthington</i>	I, XIII, XXIII	l w	
	<i>S. cubana</i>	I, XIII, XXIII	z ₁₉	
	<i>S. orientalis</i>	XVI	k	e, n, z ₁₄
	<i>S. huttlingfoss</i>	XVI	b	e, n, x

a given drug, is a consequence of antibiotic therapy that must always be borne in mind. There are on record a number of ward epidemics of streptococcal infection of burns and wounds caused by a drug-fast strain of *Str. pyogenes* (see Colebrook *et al.* 1944); but, though there was in each case ample opportunity for the therapeutic induction of drug-fastness in the epidemic strain, no proof that it had become drug-fast under these conditions was in fact forthcoming. (See also Damrosch 1946, Hartman and Weinstein 1948).

The changing fortunes of *Staph. aureus* during recent years provides an instructive example.

In 1944 Spink and Vivino noted in the U.S.A. an increasing frequency of sulphonamide resistant cocci; and a number of observers recorded the replacement of penicillin-sensitive by penicillin-resistant staphylococci in war wounds (Roy and Greenberg 1945, North *et al.* 1946, Harley *et al.* 1946). North and Christie (1945) found in one hospital where penicillin was being used a higher proportion of penicillin-resistant cocci than in a hospital where it was not. Among *Staph. aureus* isolated in certain hospital communities, Barber (1947, Barber and Whitehead 1949) records that 14 per cent. were resistant in 1946, 31 per cent in 1947 and no fewer than 37 per cent in 1948. Similar increases were observed in other countries, associated with the introduction of the general use of antibiotics—particularly with penicillin, but to some extent with streptomycin and the broad-spectrum antibiotics. The high frequencies of resistant cocci are associated with hospital wards (as compared with out-patient clinics and out-of-hospital communities); with the use of antibiotics in the wards; and with the length of stay of patients and staff in hospital. Moreover, resistant strains are found both in healthy carriers and infective lesions. The process is apparently a weeding-out of penicillin-sensitive strains by the antibiotic, leaving the resistant strains, which are penicillinase-producers, in possession of the field. There is no good evidence of induction of resistance. In a number of the surveys, the resistant strains that remain are restricted to a relatively small number of phage types (Barber *et al.* 1949, Rountree and Thomson 1949, Boe and Vogelsang 1949, 1951, Forbes 1949, Biegelman and Rantz 1950, Cairns and Summers 1950, Rountree and Barbour 1950, 1951, Erlanson 1951, Elwood 1951, Lowbury *et al.* 1952, Clarke *et al.* 1952, Vogelsang and Boe 1952, Prissick 1953, Finland and Haight 1953, Forfar *et al.* 1953, Spink 1954).

The use of an antibiotic has clearly produced communities in which resistant forms prevail among healthy carriers and among infected patients, irrespective of individual treatment with the antibiotic. The existence of two or more antibiotics that attack the bacterium without inducing mutual cross-resistance is perhaps the best safeguard against the dangers of such a situation.

Another disturbing though relatively infrequent, result of antibiotic therapy is the replacement of one infection by another. The suppression of mouth bacteria by oral penicillin infections were transformed into infections with the penicillin-resistant *Staph. aureus*. The suppression of mouth bacteria by oral penicillin

flora in the infected tissue. But, as Garrod (1951) points out, the fact there is an initial equilibrium maintained by natural antibiotic among the infecting strains; the antibiotic might be stimulating the growth of the new infection, as when acute *Ps. pyocyanea* infection of the urinary tract appears in endocarditis patients treated with penicillin (see also Stanley 1947). The "replacement" or newly appearing infection may be very severe. Finland and his colleagues drew attention to staphylococcal infection of this kind: staphylococcal pneumonia replacing pneumococcal; and urinary tract infections, "staphylococcal dysentery," and "staphylococcal scarlet fever" arising during

THE SALMONELLA TYPES (SPECIES)—*Concluded*

Immunologic Types		Antigenic Formulae*		
		Somatic Antigens	Flagellar Antigens	
Group	Species		Phase 1	Phase 2
Γ (Cont.)	<i>S. ganinara</i>	XVI	d	1, 7
	<i>S. scintes</i>	XVI	k	1, 2, 3
	<i>S. kirkee</i>	XVII	b	1, 2
	<i>S. cerro</i>	XVIII	Z ₁ , Z ₂ , Z ₃	
	<i>S. memphis</i>	XVIII	k	1, 5
	<i>S. minnesota</i>	XXI, XXVI	b	e, n, x
	<i>S. tel aviv</i>	XXVIII	y	e, n, z ₁₃
	<i>S. pomona</i>	XXVIII	y	1, 7
	<i>S. hormaeche</i>	XXIX (Vi)	Z ₁₀ , (Z ₁₁)	
	<i>S. ballerup</i>	XXIX, (Vi)	Z ₁₄	
	<i>S. urbana</i>	XXX	b	e, n, x
	<i>S. adelaide</i>	XXXI	f, g	
	<i>S. mouschauti</i>	XXXI	m, t	
	<i>S. innerness</i>	XXXVIII	k	1, 6
	<i>S. champagne</i>	XXXIX	k	1, 5
	<i>S. waycross</i>	XLI	Z ₄ , Z ₂₁	

one another in several respects. The flagellar antigen is the more unstable and is destroyed by boiling and by exposure to alcohol or weak acid, somatic antigen, on the other hand, is stable to boiling, alcohol and acid. Cultures on phenol agar (0.1 per cent) of bacteria normally containing both H and O antigens are found to contain only O antigens, the formation of flagellar antigen having been suppressed. H antigen reappears immediately on cultivation on nutrient agar. In the agglutination reaction, bacteria lacking flagellar antigen are characteristically clumped in a finely granular precipitate (O agglutination), while bacteria containing flagellar antigen are agglutinated in a coarse, flocculent precipitate (H agglutination).

The H and O agglutination titer of an antiserum may be determined by the use of H and O antigens in the agglutination test. H antigen is commonly prepared by adding an equal volume of formal (0.6 per cent formalin) saline to an eighteen to twenty-four-hour broth culture. In the preparation of somatic antigen the flagellar component is destroyed by treatment with alcohol, the growth from an eighteen- to twenty-four-hour agar slant culture is emulsified in 1 to 2 ml. of absolute alcohol, heated at 60° C. for one hour, centrifuged and the sediment suspended in 0.5 to 1 ml. saline. It may be used for slide agglutination or appropriately diluted for macroscopic agglutinin titrations.

These two types of antigen are immunologically independent, and the immunization of an animal with a microorganism containing both results in the production of antibodies to both. There is, however, a marked difference in titer, for the O antibody titer is generally much lower than that of the H antibody; in the writer's laboratory antisera having H titers of 1:20,000 to 1:50,000 have shown O titers of 1:2,000 or less.

Antiscarlatinal (antitoxic) and the ordinary type of antistreptococcal serum, which have already been described in relation to the treatment of scarlet fever and puerperal sepsis (Chapter 66), have on the whole given disappointing results in cases of septicæmia.

Active Immunization.—Examination of the blood serum of man and animals has shown the presence in small amounts of natural antitoxin to staphylococci (see Bryce and Burnet 1932, Parish, O'Meara, and Clark 1934, Murray 1935, Nélis and Poncelet 1935, Gernez and Pannequin 1937, Weiss 1939). In normal human adults and new-born babies the antitoxin titre expressed in terms of international units is generally about 0.25–0.75 unit per ml. In patients suffering from superficial staphylococcal infections, such as acne, blepharitis, furunculosis, and sycosis, the average titre is very slightly higher or practically unaltered. In cases of carbuncles the titre is often slightly raised, and in cases of chronic osteomyelitis it is generally high, though not always so (see Blair and Hallman 1935–36), and may reach 15 units or more per ml. Experimentally and clinically it has been found that treatment of animals and patients suffering from chronic staphylococcal infections with formolized toxin—toxoid—but not with staphylococcal vaccines, is often followed by a rise in the antitoxin titre and considerable improvement in the local condition; though there seems to be no definite relationship between the absolute titre attained and the retrogression of the infection. The preparation of the toxoid requires care, and strict precautions must be taken to ensure its innocuity and its antigenic potency (Dolman and Kitching 1935, 1936, Smith 1936, 1937, Farrell 1941). The dosage recommended by different workers varies somewhat. Dolman (1933, 1935) starts with a dose of 0.05 ml., increases by 0.05 ml. weekly, till at the eighth injection 0.5 ml. is given. If a second course of injections is needed, it is probably wise to rest the patient for a month or two. There seems to be fairly general agreement now that toxoid is of considerably more value than vaccines of whole staphylococci. Furunculosis and staphylococcal skin lesions appear to be particularly benefited by toxoid treatment. The results have been disappointing in chronic osteomyelitis (Blair and Hallman 1936, Buchman 1937). In cases of acne, staphylococcal toxoid may well be combined with a vaccine of *C. acnes*. (For reports on treatment with toxoid see Dolman 1933, 1935, Dolman and Kitching 1935, Connor and McKie 1934, Parish, O'Meara, and Clark 1934, Murray 1935, Whutby 1936, Mercier 1937, Ramon *et al.* 1946, Bocage *et al.* 1947, MacDonald and Taylor 1951.)

Vaccines of *Bact. coli* and of *Proteus vulgaris* are sometimes of use in urinary infections. For further discussion of the value of vaccine treatment the reader is referred to the monograph by Dudgeon (1927).

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THE SALMONELLA TYPES (SPECIES)—Continued

Immunologic Types		Antigenic Formulae*		
		Somatic Antigens	Flagellar Antigens	
Group	Species		Phase 1	Phase 2
E-1	<i>S. london</i> (L II)	III, X, XXVI	l, v	1, 6
	<i>S. givc</i>	III, X, XXVI	l, v	1, 7
	<i>S. uganda</i>	III, X, XXVI	l, z ₁₁	1, 5
	<i>S. anatum</i>	III, X, XXVI	e, h	1, 6
	<i>S. muenster</i>	III, X, XXVI	e, h	1, 5
	<i>S. nyborg</i>	III, X, XXVI	e, h	1, 7
	<i>S. vejle</i>	III, X, XXVI	e, h	1, 2, 3
	<i>S. maleagris</i> ✓	III, X, XXVI	e, h	1, w
	<i>S. shangai</i>	III, X, XXVI	d	1, 5
	<i>S. zanzibar</i>	III, X, XXVI	k	1, 5
	<i>S. amager</i>	III, X, XXVI	y	1, 2, 3
	<i>S. lexington</i>	III, X, XXVI	z ₁₀	1, 5
	<i>S. wellereden</i>	III, X, XXVI	r	z ₆
	<i>S. orion</i>	III, X, XXVI	y	1, 5
	<i>S. butantan</i>	III, X, XXVI	b	1, 5
	<i>S. saipan</i>	III, X, XXVI	z ₄	1, 6
E-2	<i>S. newington</i>	III, XV	e, h	1, 6
	<i>S. selandia</i>	III, XV	e, h	1, 7
	<i>S. new brunswick</i>	III, XV	l, v	1, 7
	<i>S. illinois</i>	[III], [XV], XXXIV	z ₁₀	1, 5
	<i>S. cambridge</i>	III, XV	e, h	1, w
E-3	<i>S. senftenberg</i>	I, III, XIX	g, s, t	
	<i>S. niloese</i>	I, III, XIX	d	z ₆
	<i>S. simsbury</i>	I, III, XIX	z ₂₇	
	<i>S. faksony</i>	I, III, XIX	i	z ₆
F	<i>S. heves</i>	VI, XIV, XXIV	d	1, 5
	<i>S. carrau</i>	VI, XIV, XXIV	y	1, 7
	<i>S. onderstepoort</i>	(I), VI, XIV, XXV	e, [h]	1, 5
	<i>S. florida</i>	(I), VI, XIV, XXV	d	1, 7
	<i>S. madelia</i>	(I), VI, XIV, XXV	y	1, 7
	<i>S. sundsvall</i>	(I), VI, XIV, XXV	z	e, n, x
	<i>S. kentucky</i>	(VIII), XX	i	z ₆
	<i>S. aberdeen</i>	XI	i	1, 2, 3
	<i>S. rubislaw</i>	XI	r	e, n, x
	<i>S. pretoria</i>	XI	k	1, 2, 3
	<i>S. solt</i>	XI	y	1, 5
	<i>S. luciana</i>	XI	a	e, n, z ₁₁
	<i>S. venezia</i>	XI	i	e, n, x
	<i>S. senegal</i>	XI	r	1, 5
	<i>S. marseille</i>	XI	a	1, 5
	<i>S. chandans</i>	XI	d	e, n, x
	<i>S. grumpensis</i>	XIII, XXII	d	1, 7
	<i>S. poona</i>	XIII, XXII	z	1, 6
	<i>S. borbeck</i>	XIII, XXII	l, v	1, 6
	<i>S. mississippi</i>	I, XIII, XXIII	b	1, 5
	<i>S. wichita</i>	I, XIII, XXIII	d	
	<i>S. hasana</i>	I, XIII, XXIII	f, g	
	<i>S. worthington</i>	I, XIII, XXIII	l, w	z
	<i>S. cubana</i>	I, XIII, XXIII	z ₁₉	
	<i>S. orientalis</i>	XVI	k	e, n, z ₁₁
	<i>S. hyttingfoss</i>	XVI	b	e, n, x

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THE SALMONELLA TYPES (SPECIES) — *Concluded*

Immunologic Types		Antigenic Formulae*		
Group	Species	Somatic Antigens	Flagellar Antigens	
			Phase 1	Phase 2
F (Cont.)	<i>S. ganymena</i>	XVI	d	1, 7
	<i>S. szentes</i>	XVI	k	1, 2, 3
	<i>S. kirkce</i>	XVII	b	1, 2
	<i>S. cerro</i>	XVIII	Z ₄ , Z ₁₇ , Z ₁₈	
	<i>S. memphis</i>	XVIII	k	1, 5
	<i>S. minnesota</i>	XXI, XXVI	b	e, n, x
	<i>S. tel Aviv</i>	XXVIII	y	e, n, Z ₁₅
	<i>S. pomona</i>	XXVIII	y	1, 7
	<i>S. hormaeche</i>	XXIX (A ₁)	Z ₂₀ , (Z ₂₁)	
	<i>S. bullerup</i>	XXIX, (V ₁)	Z ₁₄	
	<i>S. urbana</i>	XXX	b	e, n, x
	<i>S. adelaide</i>	XXXV	f, g	
	<i>S. montevideo</i>	XXXV	m, t	
	<i>S. enteritidis</i>	XXXVIII	k	1, 6
	<i>S. champagne</i>	XXXIX	k	1, 5
	<i>S. newcross</i>	XLI	Z ₄ , Z ₂₁	

one another in several respects. The flagellar antigen is the more unstable and is destroyed by boiling and by exposure to alcohol or weak acid, somatic antigen, on the other hand, is stable to boiling, alcohol and acid. Cultures on phenol agar (0.1 per cent) of bacteria normally containing both *H* and *O* antigens are found to contain only *O* antigens, the formation of flagellar antigen having been suppressed, *H* antigen reappears immediately on cultivation on nutrient agar. In the agglutination reaction, bacteria lacking flagellar antigen are characteristically clumped in a finely granular precipitate (*O* agglutination), while bacteria containing flagellar antigen are agglutinated in a coarse, flocculent precipitate (*H* agglutination).

The *H* and *O* agglutination titer of an antiserum may be determined by the use of *H* and *O* antigens in the agglutination test. *H* antigen is commonly prepared by adding an equal volume of formol (0.6 per cent formalin) saline to an eighteen- to twenty-four-hour broth culture. In the preparation of somatic antigen the flagellar component is destroyed by treatment with alcohol; the growth from an eighteen- to twenty-four-hour agar slant culture is emulsified in 1 to 2 ml. of absolute alcohol, heated at 60° C for one hour, centrifuged and the sediment suspended in 0.5 to 1 ml. saline. It may be used for slide agglutination or appropriately diluted for macroscopic agglutinin titrations.

These two types of antigen are immunologically independent, and the immunization of an animal with a microorganism containing both results in the production of antibodies to both. There is, however, a marked difference in titer, for the *O* antibody titer is generally much lower than that of the *H* antibody, in the writer's laboratory antisera having *H* titers of 1:20,000 to 1:50,000 have shown *O* titers of 1:2000 or less.

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SPECIFIC AND NON-SPECIFIC FLAGELLAR ANTIGENS. The flagellar antigen is, in turn, of dual nature. One kind, designated as *specific flagellar antigen*, is individualistic and contributes in no small part to the immunological identity of a given Salmonella species. The other, termed *non-specific flagellar antigen*, is made up of a limited number of components which are frequently shared by the various Salmonellas and hence contribute to the immunological relationship between the species of these bacteria. The specific flagellar antigens are arbitrarily designated by lower case letters (the choice was unfortunate since the more recently discovered antigens are designated z_1 , z_2 , z_3 , etc.), and the non-specific ones by Arabic numerals.

The antibodies to these antigens behave independently; in the presence of homologous H and O antibodies heated antigen agglutinates in the characteristic fine granular form, while with unheated bacilli coarse flocculation occurs, mixed, within the range of the O antibody titer, with the granular clumps. The antibodies may be selectively absorbed by appropriate antigens, and such absorbed sera are sometimes designated *mono-specific sera*. By the absorption technique using homologous, heterologous or treated antigens, the antigenic components of the bacterial cell may be determined, and through manipulations of this kind, termed *antigenic analysis* (p. 300), White,⁴ Kauffmann⁵ and others have elucidated the complex antigenic structure and interrelationships of the bacteria of the Salmonella group.

Antigenic Formulae. By identifying these antigenic components it becomes possible to write a formula that describes the immunological character of a given species or strain. Such formulae for antigenic structures are commonly used and are illustrated in the accompanying table.

Classification. While the bacteria given the common generic name Salmonella are obviously closely related to the other members of the enteric group, the interrelationships of the varieties or types of these bacilli to one another is not altogether clear. As indicated above, prior to the emphasis upon immunological character which has been marked since the 1920's, bacteria of this group were separated from one another on the basis of physiological characters. In the more commonly occurring varieties, at least, there is a marked association between biochemical properties and immunological constitution. It would seem neither justified nor desirable to subordinate completely the physiology of these bacteria to their antigenic character.

However this may be, the extent to which antigenic composition should be allowed to determine Salmonella species is open to question. At first it was customary to consider a new immunologic type as a new species, and many of these were given place names. As a consequence a great number of species have been described and continue to be, at the rate of several every year, and at the present time the situation has become absurd. Simplification is obviously essential, but what form it will take is as yet not clear.

Variation. Possibly because the antigenic structure of the Salmonella group is known in considerable detail, two general types of variation may be differentiated. One of these is a type of fluctuating, completely reversible immunological variation known as *phase variation*, and the other the S-R dissimilation known to occur in practically all bacteria.

⁷ Cf. Kauffmann Ztschr. f. Hyg u. Infektionskr., 1937, 120 177.

Most of the strains isolated either produced only a green coloration on blood agar, and were usually classed as *Str. viridans*, or were non-hæmolytic. A few hæmolytic strains were also isolated.

Lesions resembling those in rheumatic infection of man have been produced in rabbits by intravenous inoculation of these various streptococci. They include endocarditis, non-suppurative myocarditis, and arthritis of all degrees of severity, sometimes associated with extensive, secondary bony changes (Poynton and Paine 1900, 1913, Cole 1904, Wächter 1908, Bracht and Wächter 1909, Coombs *et al.* 1912, Jackson 1912, Rosenow 1914, Topley and Weir 1921, Birkhaug 1927, Small 1927, Gross *et al.* 1929, Clawson 1930, Moon and Stewart 1931). But although the analogy between experimental rheumatism in the rabbit and the natural infection in man is close, it is not exact. This applies particularly to the myocardial lesions. Aschoff (1904) described in detail characteristic nodular collections of cells in rheumatic myocarditis, and his observations have been fully confirmed by later workers (Geipel 1905, 1909, Fraenkel 1912). In spite of a certain resemblance between some of the myocardial lesions in rabbits and the typical Aschoff nodule (Coombs *et al.* 1912, Jackson 1912), it is very doubtful whether the rabbit lesions can be regarded as pathognomonic (Wächter 1908, Bracht and Wächter 1909, Topley and Weir 1921, Gross *et al.* 1929).

However closely experimental streptococcal infection in the rabbit simulates acute rheumatism in man, it is quite certain that the ability to produce these lesions on intravenous inoculation cannot be regarded as evidence that a particular variety of streptococcus is ætiologically related to the human disease; for many of the streptococcal strains which have produced the most characteristic lesions in the rabbit were derived from sources quite unconnected with rheumatic infection, and some of them were actively hæmolytic (Cole 1904, Jackson 1912, Topley and Weir 1921). It is clear that experimental rheumatism in the rabbit is a characteristic reaction of that animal species to the intravenous inoculation of a variety of different streptococci.

To summarize: it is seldom possible to cultivate any bacteria from the local lesions during life; blood cultures remain sterile in a large proportion of cases during the febrile stage of the disease; but, in either case, when any bacterium has been isolated, it has almost invariably been a streptococcus; cultures obtained at autopsy have, as would be expected, given growth in a higher proportion of cases, though by no means in all, and here again a streptococcus has been the predominating organism; and, though inoculation of experimental animals with streptococci may incite lesions simulating those of rheumatic infection, the variety of mixing streptococci is too great to permit conclusions about a single agent in the human disease.

Before leaving the problem of the possible relation of these heterogeneous strains of streptococci to rheumatic diseases it should be noted that many observers have suggested the possibility that some forms of rheumatism, at least, are allergic manifestations. This view was, for instance, developed by Zinsser and Yu (1926) and receives some support from the relative frequency of allergic skin reactions to killed cultures of streptococci of various types (see, for instance, Swift, Wilson and Todd 1929).

Hypersensitiveness to the antigens of various streptococci is also common in non-rheumatic subjects (Derick and Fulton 1931). However, when products of *Str. pyogenes* are used for the tests, as many observers have recorded, rheumatic subjects prove to be more frequently sensitive than non-rheumatic subjects (see Green 1942*d*). Thus Gibson, Thomson and Stewart (1933) recorded a higher proportion of skin reactions to extracts of hæmolytic streptococci among 140 rheumatic cases than among 145 controls, whereas there was no significant difference between the skin reactions of these groups to extracts of non-hæmolytic streptococci. On the other hand the Dick reaction was positive in only 16 per cent. of the rheumatic cases as compared with 28 per cent. of the controls. The greatest

Phase Variation The *H* antigens of certain *Salmonella* types can be separated into relatively stable components. This was first demonstrated by Andrewes⁸ and may be shown very simply by plating out such a type and carrying out slide agglutination of individual colonies with monospecific antiserum. About half the colonies will be found to contain one kind of antigen and about half the other. Apparently the individual bacterial cells do not contain both kinds of *H* antigen but are of two kinds in this respect.⁹ This immunologic character breeds true to only a limited extent, for if a colony is picked and subcultured in broth, platings of successive broth cultures will show a rapid reversion to the 50:50 ratio within a very few transfers. This type of immunological variation is called *phase variation* and the transitory immunological types were originally called the *specific phase*, i.e., that characterized by the presence of specific flagellar antigens, and the *non-specific phase*, i.e., that in which the non specific antigens occurred. This division is not as sharp as once thought, however, and now these phases are commonly referred to as *phase 1* and *phase 2* respectively.

The *Salmonella* types that exist in two immunological phases are termed *diphaseic*, while those that exist in only one phase, which may be either phase 1 or phase 2, are called *monophaseic*. There is reason to believe that the monophaseic types are potentially diphaseic, and possibly they are degenerated diphaseic forms, for it has been shown that *S. paratyphi A*, a monophaseic type stable in phase 1, can be induced to form phase 2 antigens by cultivation in antiserum specific to the phase 1 antigens. Conversely, diphaseic types can be stabilized in one phase by cultivation in the presence of antiserum to the antigens of the other phase. By this means *Salmonella* "species" may be transformed, Edwards, Moran and Bruner,¹⁰ for example, have so transformed *S. sinnsbury* into *S. senftenberg*. Furthermore, "new" species may be created from monophaseic types by suppression of flagellar antigen through cultivation in the presence of monospecific antiserum, with the appearance of a hitherto unknown flagellar phase. Such an induced antigen in *S. minnesota* was later found to occur naturally.¹¹ Phase variation does not occur, of course, in *S. pullorum* which is non motile and contains no flagellar antigen.

Phase variation is somewhat more complex than this, however, and three types have been described. The first is the specific-non-specific phase variation of Andrewes⁸ in which a specific antigen ordinarily occurring in phase 1 is linked with the non specific antigens of phase 2. The second is the so-called $\alpha\beta$ phase variation described by Kauffmann and Mitsui¹² in which an antigen occurring in phase 1 is linked with the antigens ϵ , η , θ , in phase 2. Five types of $\alpha\beta$ phase variation have been reported by Edwards and Bruner.¹³ Lastly, there is a type of phase variation which has not been named in which the antigens of both phases are commonly found in phase 1.¹⁴

It is not clear whether this type of immunological variation occurs gen-

⁸ Andrewes Jour. Path. Bact., 1922, 25:505.

⁹ Andrewes Jour. Path. Bact., 1922, 25:505.

¹⁰ Edwards, Moran and Bruner Jour. Hyg., 1938, 38:716.

¹¹ Edwards and Bruner Jour. Hyg., 1938, 38:716.

¹² Kauffmann and Mitsui Jour. Hyg., 1938, 38:716.

¹³ Edwards and Bruner Jour. Hyg., 1938, 38:716.

¹⁴ Edwards and Bruner Jour. Hyg., 1938, 38:716.

in 8 of 9 fatal cases of rheumatic fever; in the cadavers of 22 non-rheumatic subjects *Str. pyogenes* was found on the valves only twice, and in these two cases blood cultures were also positive. Collis (1939) examined only rheumatic cases, and found hæmolytic streptococci in 14 of 15 tonsils, 13 of 27 cervical or mediastinal lymph nodes, and 22 of 42 heart valves cultured (see also Thomson and Innes 1940).

In the Canadian army about 4 per cent. of persons suffering from streptococcal infection of the respiratory tract are said to have developed acute rheumatism. Of throat cultures taken from 46 patients, 14 yielded no streptococci, 2 yielded *Str. viridans*, and 30 yielded hæmolytic streptococci, all of which, with one possible exception, belonged to Group A (Feasby 1944). The epidemiology of acute streptococcal infections of the upper respiratory tract among the armed forces in the United States during the 1939-45 war provided further evidence of this association. Thus Rantz and his colleagues (1945, 1947) observed 15 cases of rheumatic fever among 410 men who had had invasive streptococcal infections, and none among 1,100 not so infected. They also found other sequelæ of infection, not obviously rheumatic, and concluded that rheumatic carditis was an extreme manifestation of a "post-streptococcal state," which might supervene in up to a quarter of the infected persons. Moreover, the incidence of the post-streptococcal state was associated with evidence of re-infection with streptococci (see also Watson *et al.* 1945, Wheeler and Jones, 1945, Quinn 1947).

The significance of the epidemiological findings is greatly increased by serological evidence of infection by *Str. pyogenes*. Todd (1932) showed that the acute rheumatic process was almost constantly associated with a wide fluctuation in the titre of antibody to the O streptolysin in the blood of the patient. This fluctuation is of a rather peculiar kind, in that the rise in titre occurs during the acute rheumatic attack, and particularly during a relapse, whereas in quiescent or recovered cases the titres are not greatly above normal. These observations were confirmed by Coburn and Pauli (1932c, 1935a, c, f).

Later observations showed that the anti-streptolysin O titre rose in scarlet fever and streptococcal pharyngitis. The response in these two diseases is at a maximum two to three weeks after their onset; that in rheumatic fever occurs rather later—4 weeks or more after the onset (Coburn 1936, Coburn and Pauli 1939b, Todd, Coburn and Hill 1939) Green (1941) gives 79 units per ml. as the average titre in healthy subjects, 263 in pharyngitis, 300 in scarlet fever, and 210 in inactive, and 444 in active rheumatic fever. There is little doubt that, as a rule, the titres rise higher and stay up longer in rheumatic fever than in more direct streptococcal infections (see, for example, Quinn and Liao 1950). However, not all rheumatic subjects exhibit the large and delayed response (Taran *et al.* 1946) and, when it occurs, it may not signify more than that rheumatic fever is a long-lasting disease (Harris and Harris 1950), or an association between rheumatic fever and exposure to streptococcal infection and re-infection (Rantz *et al.* 1951, Quinn *et al.* 1951).

There is other evidence of a serological response to the antigens of *Str. pyogenes*. Agglutinating and complement-fixing antibodies for hæmolytic streptococci (Coburn and Pauli 1932c, Thulin 1948, Liao 1949), antifibrinolysin (antistreptokinase) (Stuart-Harris 1935, Lichty 1941, Mote and Jones 1941, Anderson *et al.* 1948), antihyaluronidase (Friou and Wenner 1947, Quinn 1948, Harris and Harris 1949, Stoppelman 1950), precipitins for group and type-specific antigens of Group A streptococci (Swift and Hodge 1936, Coburn and Pauli 1939b, Mote and Jones 1941, Rothbard *et al.* 1948), and complement-fixing antibodies for nucleoprotein and other fractions of the streptococcus (Harris 1949) in general resemble streptolysin O in occurring more abundantly, and rather later, in rheumatic fever than in other streptococcal diseases.

These serological facts are certainly good evidence of the participation of the antigens of *Str. pyogenes* in the rheumatic syndrome. But there is no consistent evidence that any one antigen is peculiarly associated with the rheumatic attack.

erally with respect to the somatic antigens. A similar variation, known as *form variation*, does occur, however. Kauffmann¹⁵ has found that of the three components of XII, designated XII₁, XII₂, and XII₃, XII₂ varies in that it is either strongly or weakly developed. Antigen VI is likewise subdivided into VI₁ and VI₂ but similar variation in these latter components has not as yet been reported.

It is to be emphasized that the immunological changes associated with phase variation are, so far as is known, normal, the bacterial strain seemingly existing in a sort of immunological equilibrium. These variations and the complete but temporary loss of flagellar antigen by cultivation on phenol agar noted above apparently bear no relation to the S-R dissociation though, as indicated, they may be induced by a method which will induce dissociation, i.e., cultivation in specific antiserum. There also seems to be some tendency to natural segregation of antigenic components. Edwards¹⁶ has observed such a segregation in the H antigens, z₃₀, z₃₁, of *S. hormaechi*; the components appeared to segregate in that for a period of time variants containing only z₃₀ occurred and later variants containing only z₃₁ were thrown off, both of which were stable and bred true. It is not unlikely that such variation occurs in nature on the one hand, and that the monophasic types represent loss variants, giving rise to a multiplicity of antigen combinations. The phylogenetic implications of the pattern of distribution of antigens within the Salmonella group and of phase variation have been discussed by White,⁴ Edwards⁸ and Bruner and Kauffmann¹⁶ but cannot be considered here.

Dissociation. S-R dissociation, similar in all respects to that known in other bacteria, occurs in cultures of these bacilli. The dissociation from smooth to rough is manifested as an alteration in colonial morphology and loss of virulence. The change is reflected immunologically as a loss of specificity of the somatic antigens, i.e., the rough forms remain motile. The specificity of these antigens is apparently determined by a polysaccharide haptene, and with the disappearance of the haptene the bacteria acquire a new and common immunologic character in the somatic antigens, while the flagellar antigens remain unchanged. A mucoid or M phase in colonial morphology has been reported by some workers which is said to be associated with the development of a new immunological specificity.

Bacteriological Diagnosis of Salmonella Infection. The differentiation of the paratyphoid fevers from typhoid fever and the determination of the etiology of gastro-enteritis caused by Salmonella is necessarily dependent on the isolation and identification of the causative microorganism.¹⁷ For isolation both enrichment culture and direct plating should be used; enrichment broth and differential selective agar plates should be inoculated simultaneously and, if the latter are negative, fresh plates can be inoculated from the enrichment culture. It is commonly observed that no single agar medium suffices, for with very few bacteria cultures may be isolated on one medium but not the others; at least two, and better three, kinds of differential agar should be used.

Two enrichment media are commonly used. Selenite-F broth contains 0.4

¹⁵ Kauffmann Jour. Bact., 1941, 41:127.

¹⁶ Edwards Jour. Bact., 1946, 51:523.

¹⁷ Isolation procedures are discussed in some detail by Littman War Med., 1943, 431.

failure of immunization with *Str. pyogenes* toxin to prevent the development of acute rheumatism (see Coburn and Pauli 1935a), we may conclude that the erythrogenic toxin plays little part in the disease process.

Even though we accept the broad indications of the streptococcal rôle in acute rheumatism it is clear that, compared with typical infections like pharyngitis or scarlet fever, the rheumatic response to the streptococcus is curiously delayed. The cause of this abnormality does not appear to lie in the organisms themselves, for they do not differ demonstrably from strains associated with other types of infection. Poverty, bad housing, urbanization, dietary deficiencies and hereditary tendencies have all been implicated as predisposing factors (see, for example, Glover 1939, 1943, Morris and Titmuss 1942, Coburn 1945, Holmes and Rubbo 1953), but are not easily interpreted in terms of a peculiar reactivity to *Str. pyogenes*. As we have seen in Chapter 54, there is little direct basis for implicating vitamin C deficiency as a major factor (but see Long 1954).

Allergy and Rheumatic Fever.—The foregoing evidence is difficult to reconcile with the view that rheumatic fever is the result of a simple chronic infection by *Str. pyogenes*, whether generalized or local. Allergy has been justifiably invoked to explain some features of the disease, but the question as to why a proportion of the persons infected with hæmolytic streptococci should react in this peculiar manner remains unanswered. Coburn (1940) suggests that the delayed reaction to the initial streptococcal infection is due to an inadequate response of the normal defence mechanisms, which permits the establishment of streptococcal foci in the tissues. The liberation of antigen from these foci results at first in allergic sensitization, and, at a later stage, in a concurrent stimulation of the antibody-forming apparatus and in acute allergic inflammation in other sensitized tissues.

The observation of Coburn and Pauli (1939a) that, in the quiescent stage after the initial pharyngitis prior to the onset of the rheumatic attack, a substance ("precipitinogen") appears in the blood, which is specifically precipitated by sera taken from the same patient or from other patients in the later acute rheumatic stage, suggested the replacement of a circulating antigen by an antibody specific for it. Wedum and Wedum (1946), however, observed this "phase" reaction in non-streptococcal conditions, and Fischel and Pauli's (1949) evidence suggests that it is due to a non-specific colloidal abnormality of the serum, and that a specific antigen-antibody reaction is not concerned.

A variant of the allergic hypothesis was proposed by Brokman, Brill and Frenzel (1937) who, on the basis of positive complement-fixation reactions of rheumatic sera with an extract of liver from a rheumatic child, suggested that the lesions of the disease were produced by "auto-antibodies" reacting with components of the host's own tissues. Cavelti and Cavelti (1945) induced auto-antibodies to kidney, muscle and connective tissue, detectable by the agglutination of collodion particles coated with the appropriate tissue extract, by injecting into rabbits or rats Group A streptococci mixed with homologous tissues. The appearance of antibodies was accompanied by lesions in the kidney, muscle or connective tissue, according to the antigen used. Cavelti (1945) also detected auto-antibodies to extract of human heart in rheumatic patients. These findings have not been confirmed by other observers (Humphrey 1948, Peck and Thomas 1949, More et al. 1949, Fischel and Pauli 1949), although Glynn and Holborow (1952) induced antibody to otherwise non-antigenic chondroitin sulphate when it was adsorbed to a streptococcal vaccine; and the animals immunized with this vaccine developed acute synovitis.

We may also note here the view that the rheumatic syndrome is due to body proteinases activated by the streptococcus (see Mirsky 1945).

Neither the allergic nature of acute rheumatic manifestations, nor the rôle of

from this disease (Dawson, Olmstead and Boots 1932b, Dawson, Olmstead and Jost 1934, Dawson and Tyson 1936, Neil and Hartung 1937). Kalbak (1947; see also Cöster 1930) records an agglutination reaction with living Group A streptococci, positive in about 80 per cent. of patients with rheumatoid arthritis, and in less than 15 per cent. of those with rheumatic fever and other streptococcal diseases. Wallis (1947), on the other hand, regards the increase in precipitin and agglutinins as a non-specific enhancement of antibodies normally present.

Waaler (1940), and Rose and his colleagues (1948) described in the sera of patients with rheumatoid arthritis a serum component that enhanced the agglutination of sheep red cells sensitized with anti-R.B.C. rabbit serum (see also Scott 1952). This component appears to be a globulin acting non-specifically as a co-agglutinin, and only fortuitously associated with the disease; it behaves like the C'4 component of complement (see Pike *et al.* 1949, 1951, Hobson and Gorrill 1952).

On the basis of positive agglutination tests with patients' sera, pleuropneumonia like organisms have been proposed as a cause of rheumatoid arthritis (Wallerstein *et al.* 1946).

Bacterial Endocarditis

Although this disease bears no immediate or necessary relationship to acute rheumatism, it is convenient to consider it in this chapter, if only because the isolation of non-hæmolytic streptococci from the blood stream in the more chronic cases of this kind has perhaps done something to confuse the issue in regard to the causation of simple rheumatic endocarditis.

The bacteria most commonly found in large proliferating and ulcerating lesions of the heart valves are the various pyogenic cocci: hæmolytic streptococci, streptococci of the *Str. viridans* and *Str. faecalis* types; pneumococci, gonococci and meningococci; *Staph. aureus*, *Staph. albus* and micrococci of various kinds. Of other bacteria, bacilli of the *Hæmophilus* group occur with the greatest frequency (see Perry 1936), though endocardial infections with members of the *Bacterium* and *Bacillus* groups, with salmonellæ, brucellæ, corynebacteria, clostridia (see More 1943), actinomyces and actinobacilli (see Blevins and MacNeal 1946), bacteroides, and so forth, have occasionally been reported.

In a number of instances, the endocarditis is apparently secondary to infection elsewhere in the body, usually by the more pathogenic organisms like *Staph. aureus*, *Str. pyogenes* or the pneumococcus. In others, the infection of the heart valves is apparently primary, and usually runs a less acute, though no less fatal course than that in the first group.

Subacute Bacterial Endocarditis.—There can be no question that this fatal disease is due in the great majority of cases to the infection of a previously damaged, or congenitally abnormal, valve with streptococci, either of the viridans or of the enterococcus type (see Moran 1938, Swain 1940, Solowey 1942); though other organisms are associated with the subacute infections, including influenza bacilli and *H. para-influenzæ* (see, for instance, Horder 1908-9, Schottmüller 1910, Kreidler 1926, Miles and Gray 1938, Khairat 1940, Goudie and Lowther 1951) and more rarely organisms such as staphylococci (Boe 1950), *Erysipelothrix* (Lawes *et al.* 1952), *Acinobacillus muris* (Petersen *et al.* 1950) and veillonellæ (Loewe, Rosenblatt and Altire-Werber 1946; see also Priest *et al.* 1947, Cates and Christie 1951).

As regards the streptococci, a few strains belong to Groups A, D, G, K and H; D and H are the commonest. The remainder have been variously grouped, both biochemically and serologically. Among these we may note "*Strept. subsp.*" (Loewe, Plummer *et al.* 1946), which is similar to the *Str. sanguis* of White and Niven (1946). Helbre and Neill

the negative agglutination tests in apparent typhoid fever are probably a result of paratyphoid infection. The only method by which typhoid and paratyphoid fever can be distinguished is isolation and identification of the causal microorganism.

Many scattered cases of paratyphoid fever have been observed, and a number of more or less extensive epidemics have been reported as due to milk and other foods, to contact with human carriers, to sewage-polluted water and similar factors. In general, the mode of dissemination of paratyphoid fever is practically identical with that of typhoid fever (p. 457).

The frequency of the paratyphoid fevers as compared with typhoid fever varies a good deal in different localities, but most hospital records give a ratio of less than 1:10. In some regions the proportion of paratyphoid to typhoid may be as high as 1:4 or even more. During the first World War the proportion of paratyphoid to typhoid reached a high point. In the British armies in France during the years 1915-1918, the diagnosed paratyphoid fevers outnumbered the typhoid cases 2:1. In civilian populations in most countries paratyphoid fevers probably amount to 5 or 10 up to 50 per cent or more of all fully diagnosed enteric cases. Very young individuals appear to be the most susceptible to Salmonella infection, and an unduly large portion, perhaps 20 per cent, of cultures are from young children.

Three different species have been commonly recognized as the cause of paratyphoid fever: *S. paratyphi A*, *S. paratyphi B* and *S. paratyphi C*. It may be noted that a mixed vaccine is commonly used in the prophylactic inoculation against typhoid fever which includes not only typhoid bacilli but *para A* and *para B* bacilli also (p. 461); *para C* is not ordinarily included.

Salmonella Paratyphi A (*Bacillus paratyphosus A*, *Bacterium paratyphosum A*, *Salmonella paratyphi*). This bacterium differs culturally from most of the other Salmonella species in its inability to ferment xylose, and it is, in addition, serologically distinct. Some outbreaks due to this microorganism have been traced to sewage-contaminated water supplies, others to food contaminated through the agency of human carriers. Paratyphoid fever due to *S. paratyphi A* is often very mild, 300 cases occurring in a United States infantry regiment without a single death.

Salmonella Paratyphi B (*Bacillus paratyphosus B*, *Bacterium paratyphosum B*, *Salmonella schottmulleri*). *S. paratyphi B* is readily differentiated from *S. paratyphi A*, but has confusing cultural and serological relations with certain Salmonella strains of the food poisoning types. As in the preceding species, the sources and modes of transmission are similar to those of typhoid fever. The source of the bacteria is generally the human carrier, but it has been reported that dogs²² have been found responsible for small epidemics and in another instance a cow²³ was responsible for cases of the disease. In the northern United States and in northern Europe infection with this species seems considerably more frequent than with *S. paratyphi A*.

Salmonella Paratyphi C (*S. hirschfeldii*). *S. paratyphi C* has been found in

²² Caspersen: Norsk Mag. f. Laegevidenskapen., 1937, 98, Forh. Norske Med. Selskab, 138, Ztschr. f. Hyg. u. Infektionskr., 1938, 120:611; Magnusson. Ztschr. f. Hyg. u. Infektionskr., 1938, 121:136.

²³ Rosgen and Schultze Gahmen Deut. med. Wchnschr., 1939, 65 1514.

Similar bacteraemia occurs after operative manipulation of other infected sites—the cervix uteri (Schottmüller 1911), the urethra (Barrington and Wright 1930), joints, tonsils and prostate (Richards 1932), the organisms found depending on the nature of the infection. Tonsillectomy approaches tooth extraction in the frequency with which it is followed by a *Str. viridans* bacteraemia (see Fischer and Gottdenker 1936, Elliott 1939). Dowling and his colleagues (1952) observed that heroin addicts were common among cases of staphylococcal endocarditis, and suggest that the cocci may have come from infected hypodermic injection sites.

It would seem, therefore, that in any person with oral or tonsillar sepsis, there is likely to be an occasional leak of bacteria into the blood stream, leading to a transitory bacteraemia, this leak being temporarily intensified as the result of operative procedure. These bacteria are, in a normal person, rapidly removed from the blood stream and cause no serious damage to the tissues; but if they come in contact with a congenitally defective heart valve, or a valve already damaged as the result of rheumatic infection, or a valve in which degenerative changes have occurred, they may set up a lasting and slowly fatal infection.

Treatment. The expectation of almost certain death from bacterial endocarditis has been greatly modified by modern developments in chemotherapy. Successful cures of the infection have been reported for sulphonamides, penicillin, streptomycin, bacitracin and the broad-spectrum antibiotics. Most of the organisms are susceptible to penicillin; and combinations of antibiotics may succeed when one alone is ineffective (see Cates *et al.* 1951). In one series of cases, Christie (1948, Cates and Christie 1951) records about 70 per cent. of apparent cures with penicillin. Prolonged and high dosage, of the order of 2×10^6 units daily for 1–6 weeks, was essential; relapses usually occurred within a month of stopping the penicillin. The choice of drug depends on the susceptibility of the infecting strain isolated from the blood. It is unsafe to rely simply on identification of the infecting species, because within a species the drug resistance may vary widely. The infecting strain should also be tested during therapy, and increases in resistance met by change in chemotherapy. Experimentally, the effect of the antibiotic appears to be a restraint of the growth of the organisms in the infected vegetation. This permits a deposition of bacteria-free fibrin on its surface, which in turn serves for invasion of leucocytes and reparative cells (MacNeal *et al.* 1945).

In man, the cures are apparently complete in the sense that all the organisms are destroyed (Geiger and Durlacher 1947); but the residual mechanical damage to the valves may result in progressive and ultimately fatal heart failure (Cates and Christie 1951, Kaplan *et al.* 1949).

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enteric fevers in parts of Asia, Africa and southeastern Europe and has been reported as an important cause of illness and death in British Guiana. Cases of endocarditis have been found to be caused by this and other suipestifer species. Although the type of disease is similar to the other enteric or intestinal forms, little is known about its epidemiology. This species is closely related biologically to the hog cholera or *cholerae-suis* strains described below.

Other species have been occasionally found: *S. barielly*, isolated from cases of mild pyrexia in India; *S. enteritidis* var. *moscow*, isolated from cases of paratyphoid fever in Russia and described by Russian bacteriologists as "Paratyphus N₂"; *S. sendai*, isolated from cases of paratyphoid fever in Japan, and *S. eastbourne*, from a paratyphoid case of Eastbourne, England. In this country *S. typhi-murium*, *S. saint paul*, *S. oranienburg*, *S. hartford*, *S. sendai*, *S. panama* and others have been found associated with enteric fevers.

Paratyphoid Gastro-enteritis. The symptoms of this type of disease are quite different from those in paratyphoid fever and comprise a more or less violent gastro-intestinal disturbance with vomiting, diarrhea, a slight rise in temperature and usually a rapid recovery. The attack may rarely pass into a septicemic infection. The descriptions of indigenous cholera or "cholera nostras" in earlier medical writings suggest this form of illness. Outbreaks of gastro enteritis have been usually reported in connection with the consumption of particular articles of food and are commonly referred to as "food poisoning." Food-borne infection of this type has been discussed elsewhere (p 273) and need not be considered further here. Several species of bacteria are known to be concerned.

Salmonella Typhi-murium (*Salmonella aertrycke*, *Bacterium aertrycke*, *Bacterium typhi-murium*). This bacterium, most commonly isolated in food poisoning outbreaks in the United States²⁴ and in Great Britain,²⁵ closely resembles *S. paratyphi* B in its cultural characteristics but can be distinguished by its ability to produce acid in tartrate medium. Before differential tests were satisfactorily worked out, *S. paratyphi* B and *S. typhi-murium* were commonly confused and both termed "para B." *S. typhi-murium* is commonly found in a variety of infections in laboratory and domestic animals and in birds, and the "*B. pestis caviae*" of some writers and Nocard's "*B. psittacosis*" are, in fact, *S. typhi-murium*. Most of the laboratory stock cultures labeled "Danysz virus" or "bacillus of mouse typhoid" are of the *typhi-murium* type, but some are *S. enteritidis*.

Salmonella Enteritidis (*Bacterium enteritidis*). Although found frequently in food-poisoning outbreaks, this bacterium is less common than *S. typhi-murium*. It closely resembles *S. typhi-murium* culturally, but is said by some to differ in that it does not ferment inositol while *typhi murium* does. The inositol fermentation is, however, frequently not clear-cut, a lowering of pH may be noted but sometimes not to a sufficient degree to warrant calling the fermentation positive. In the writer's experience, the inositol fermentation is variable in both species. The two may be separated by serological means.

Salmonella Cholerae-Suis (*Bacterium suipestifer*, *Bacterium cholerae-suis*, the Hog Cholera Bacillus, American Suipestifer). This *Salmonella* is a mem-

²⁴ Jordan Jour. Prev. Med., 1929, 3, 279

²⁵ Savage and White Med. Res. Council Spec. Rept. Ser. No. 92, 1925.

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ber of a group of closely related bacteria, called the *suipestifer* group, which also contains *S. paratyphi* C or Eastern type, as noted above, *S. cholerae-suis* var. *Kunzendorf* or European type, and the Glasser-Voldagsen type, which is comprised of two species, *S. typhi-suis* and *S. typhi-suis* var. *Voldagsen*. These species may be differentiated from one another by a combination of cultural and serological methods. *S. cholerae-suis* formerly predominated in the United States, but now the *Kunzendorf* variety is found more often. This species has been implicated in outbreaks of paratyphoid gastro-enteritis, although to a much lesser extent than either *S. typhi-murium* or *S. enteritidis*. *S. typhi-suis* appears to be purely an animal (pig) pathogen, but several cases of human infection with the *Kunzendorf* variety have occurred in the United States. According to Eschweiler, Wahlin and Snow²⁶ eighty-six cases of human infection with bacteremia have been reported in this country.

Other *Salmonella* species are less commonly implicated in human infections. *S. thompson* and *S. newport*, both related to the *suipestifer* group immunologically, have been observed a few times and other species but once; in fact, a number of the new *Salmonella* species described in recent years have been isolated from food implicated in outbreaks of enteritis.

The distinction between the "food-poisoning" strains of paratyphoid bacilli and the strains that cause slow typhoid-like fever does not seem to be a sharp one. In rare instances acute gastro-enteritis has been traced to *S. paratyphi* B, and, while illness caused by the food-poisoning bacilli is ordinarily followed by prompt recovery, fatal cases of generalized infection with bacteremia sometimes occur. In general, however, *S. paratyphi* A, *S. paratyphi* B and *S. paratyphi* C are found in the continued fevers; *S. typhi-murium*, *S. enteritidis* and, more rarely, *S. cholerae-suis* in acute gastro-enteritis. *Salmonellae* of the *suipestifer* group appear to be more invasive when infecting man than most of the other species, and consequently are more often found in bacteremia and other kinds of tissue infection.

Pathogenicity for Lower Animals. *Salmonella* infection of rodents is quite common; *S. typhi-murium* and *S. enteritidis* cause infections of rats and mice, and these animals may become healthy carriers of the bacilli, a point of importance in connection with the epidemiology of food-poisoning outbreaks. *S. typhi-murium* infection is by far the most common in the United States, and *S. enteritidis* less so than generally supposed. Preparations of "rat virus" or "Ratin" consist of these bacteria and are supposed to initiate an epidemic of disease in the rat population and hence destroy it. The use of such preparations is to be condemned, for not all the rats are killed and many of the survivors become healthy carriers, there is, in addition, the danger involved in leaving preparations of virulent bacilli about the home.

Salmonella infection of the horse is also quite common. Infectious abortion of mares is caused by a specific microorganism, *S. abortus equi*, which has not been found in other animals. Man is only rarely infected. *S. typhi-murium* has also occasionally been reported in horses. Abortion in sheep has been attributed to a member of the *Salmonella* group, *S. abortus ovis*. *Salmonella* is occasionally observed in a variety of other animals.

Birds are quite commonly infected with members of the *Salmonella* group.

²⁶ Eschweiler, Wahlin and Snow. Ann. Int. Med., 1944, 20:275.

CHAPTER 69

ENTERIC INFECTIONS

AMONG the clinical records left by medical writers from Hippocrates onwards, we have little difficulty in recognizing cases which can, with reasonable certainty, be identified as instances of enteric fever. The separation of this type of infection from the mass of continued fevers was, however, a slow process; and it was only during the first half of the 19th century that typhoid or enteric fever finally emerged as a recognizable clinical syndrome from among the mass of continued fevers with which it had previously been confused. Various differences in behaviour between typhoid fever and the prevalent typhus, gaol, or famine fever had indeed been noted at much earlier dates. Among these pioneers were Willis (1659) and Huxham (1739); but their descriptions were not sufficiently detailed or complete to carry general conviction. As noted by Creighton (1894), the final recognition of enteric fever in this country resulted from the elaborate analysis of the symptoms of the different types of continued fever carried out by Sir William Jenner between 1849 and 1851. In Germany, the difference between typhus and typhoid was clearly recognized by Schoenlein (1839), under the names "*Typhus exanthematicus*" and "*Typhus abdominalis*," which have maintained their position in German literature. In France, the observations of Prost (1804), and particularly of Petit and Serres (1813), afforded what was probably the first accurate description of the intestinal lesions; though many earlier records of post-mortem findings are in existence. These observations were confirmed and extended by Bretonneau (1826), Louis (1829), and Chomel (1834)—(see Gay 1918).

The most striking contribution to our knowledge of the natural history of typhoid fever, before the opening of the bacteriological era, is undoubtedly that made by William Budd (1856, 1873) of North Tawton in Devon. Budd had studied under Louis at the La Pitié hospital, and was therefore in a position to identify typhoid fever with considerable confidence. He insisted on its spread by contagion, on the reproduction of the specific poison within the living body, on the excretion of the infective material in the faeces, on the spread of the disease through the family circle by the tainted hands of those who waited on the sick, on the part played by the and on the destruction of afford an admirable example of the value of accurate observations by a practitioner carried out in the spirit of the field naturalist

With the description of the typhoid bacillus by Eberth in 1880, and its isolation by Gaffky in 1884, it became possible to attack the problems of enteric infection by the methods devised by the bacteriologist. The subsequent isolation and study of the various species of paratyphoid bacilli has shown that enteric fever, though

Epidemics due to *S. typhi-murium* sometimes cause great destruction among canaries and other songbirds. Two barnyard diseases of great economic importance are due to specific *Salmonella* types: the bacillary white diarrhea of chicks caused by *S. pullorum*; and fowl typhoid caused by *S. gallinarum* (or *S. sanguinarum*). *S. pullorum* may survive in the ovaries of the fowls that recover from infection; diseased chicks may develop from the infected ova and communicate the disease to initially healthy members of the flock. Rare cases of human infection with *S. pullorum* have been reported and it has been associated with epidemic food borne gastro-enteritis in some instances

4th week refer to cases which were febrile at that period, since such patients would naturally be selected for investigation. In any given case, it will usually be found that the decline of fever is associated with the disappearance of bacteria from the blood. In severe cases the bacteremia may not reach its peak till the 3rd week

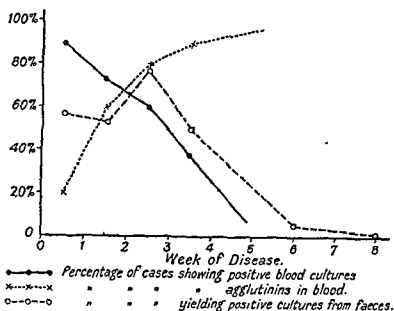


FIG. 286.

cross somewhere about the end of the 2nd week. Before that time, the bacilli are isolated more frequently from the blood than from the faeces; after it, the position is reversed. In attempting deductions from such figures as these, we must remember that differences in technique may have an important influence on our results. There are certain technical difficulties associated with blood culture, and with the isolation of pathogenic bacteria from the faeces; but they are different in kind, and the technical error involved may be quite different in degree. The significant point is the decreasing frequency of detectable bacteremia, and the increasing frequency of *Salm. typhi* in the intestine, during the first 2 to 3 weeks. This clearly suggests that the main line of invasion during this period is from the blood stream to the intestine, not the reverse. As Fig. 286 indicates, the bacilli do not disappear from the intestine so quickly or so completely as from the blood. There is a lag which is fairly constant over the early part of the falling frequency curve but which becomes more pronounced later. A considerable proportion of cases are still excreting typhoid bacilli at a time when positive blood cultures can no longer be obtained, and some cases continue excreting well into convalescence or beyond it.

Our figures for the frequency of agglutinins in the blood serum of the patient are based mainly on the results obtained with formalized broth cultures, which react with the H (flagellar) agglutinins (Dreyer *et al* 1915, 1916, 1917). During the 1st week about 20 per cent. of the cases show the presence of these agglutinins. The curve then rises sharply, crossing the blood-culture curve just before the end of the 2nd week, and still rising attains a value of 90 per cent or over by the 4th week. After this it remains at a high level for some weeks (Figs 263, 264, p. 1278). We have less information on the behaviour of the O (somatic) agglutinins, but such as we have suggests that the general form of the curve resembles that for the

of the disease (Shaw and Mackay 1951); and in cases that terminate fatally as the result of the primary infection, apart from the secondary accidents of haemorrhage and perforation, its intensity usually increases until death (Jochmann 1914).

The frequency of *Salm. typhi* in the faeces, on the other hand, increases from the 1st to the 3rd week and then falls somewhat slowly. The frequency curves for blood and faeces

THE ENTERIC BACILLI: THE TYPHOID BACILLUS

The infectious nature of typhoid fever was apparent in 1856 to William Budd, who, on the basis of epidemiological evidence, suggested that the disease was transmitted by sewage-contaminated water and that the source of the infectious material was human feces. The typhoid bacillus, however, was discovered in 1880 by Eberth in the mesenteric glands and the spleen of persons dying from typhoid fever. In 1884 Gaffky succeeded in growing Eberth's bacillus on culture media. Acceptance of this microorganism as the etiologic agent of typhoid fever, however, was delayed because typhoid fever could not be reproduced in experimental animals. The immunological aspects of typhoid fever provided strong ancillary evidence, and, in the course of time, Koch's third postulate has been fulfilled by infections in man arising from laboratory accidents.

Morphology and Staining. The typhoid bacillus closely resembles the other enteric bacteria and exhibits no distinctive morphological characters. The microorganism is a short, plump rod, ranging, as a rule, from 1 to 3.5 μ in length and from 0.5 to 0.8 μ in breadth. In smears from agar cultures the shorter forms predominate, while longer bacilli are generally observed in liquid media. It may be noted that the typhoid bacilli present in the urine of urinary carriers are frequently in the form of long filaments. This bacterium is actively motile by peritrichous flagella and generally possesses a greater number of flagella (12 to 14) than the colon bacillus (6 to 10). Spores are not formed, and the cell inclusions taken by Gaffky and others to be spores were probably either vacuoles or metachromatic granules. Capsules are not formed.

Upon agar and gelatin media the colonies of the typhoid bacillus closely resemble those of the colon bacillus and are equally variable in appearance. The "maple-leaf" appearance, arising from the irregularly notched margins and often termed typical, is by no means always seen, and the colonies are frequently round, smooth, bluish white, translucent and slightly raised. The true typhoid bacillus is never pigmented; although there have been a number of reports¹ of the isolation of yellow pigment-forming varieties, these are not true typhoid bacilli.² Typical cultures of the typhoid bacillus grow upon the surface of acid potato, but the growth is thin, moist and colorless, and forms the so-called "invisible film" which is strikingly unlike the profuse brownish growth of the typical colon bacillus. On pieces of potato with an alkaline reac-

¹ Grossmann: *Centralbl. f. Bakt.*, I, Orig., 1933, 129-508, Castro. *Deut. med. Wchnschr.*, 1934, 60-1014, Dresel and Herbert: *Arch. Hyg. u. Bakt.*, 1938, 120 286, Rotenburg: *Ztschr. Mikrobiol. Epidemiol. Immunitätsforsch. (U. S. S. R.)*, 1939, No. 5, 61.

² Cruickshank: *Jour. Hyg.*, 1935, 35.354.

in that reported by Dudgeon (1908), *Salm. typhi* has been isolated from the gall-bladder in a case of cholelithiasis, in the absence of any history of typhoid fever. The literature contains many instances of a similar kind (see Ledingham and Arkwright 1912, Gay 1918).

The mechanism by which the gall-bladder becomes infected has been studied experimentally in rabbits. Blachstein (1891) found that bacilli could be isolated from the gall bladder of these animals for days or weeks after an intravenous inoculation of living culture. Richardson (1899) recorded similar observations, and noted that some of the rabbits developed gall-stones, an observation which has been repeatedly confirmed (Gay 1918). The bacilli may reach the gall-bladder very rapidly after intravenous injection, according to Blumenthal (1910) within 10 minutes. They may persist in this situation over long periods (Morgan 1911, Johnston 1912, Gay and Claypole 1913). Weinfurter (1915) records an instance in which they were still present after 9 months.

It is generally believed that the bacilli reach the gall-bladder from the blood by travelling from the liver capillaries to the bile canaliculi, and thence down the bile ducts (Doerr 1905, Lemierre and Abram 1907, Nichols 1916). It has, on the other hand, been stated (Koch 1909, Chiarolanza 1909) that they rapidly reach the gall-bladder even after ligation of the cystic duct, and that nests of bacilli may be found in the capillaries of the gall-bladder wall, suggesting that they pass directly into the viscus by this route. It is probable, as suggested by Gay (1918), that either route may be traversed.

The picture that we have just given of the distribution of typhoid bacilli in the blood, faeces, and tissues is based on results obtained by a technique which we know to be considerably inferior to that now at our command. It may well be that the frequency of typhoid bacilli in the intestine during the early stage of the disease has been considerably underestimated. That this is true of paratyphoid B fever, the observations of Glass and Wright (1937) leave no doubt. Using a combination of tetrathionate broth for preliminary enrichment, followed by plating on eosin brilliant green agar, these workers were able, during the investigation of a sharp outbreak of paratyphoid fever at Liverpool, to isolate the bacilli from the faeces of about 83 per cent. of patients in the first week, 94 per cent. in the second, and 88 per cent. in the third. Had more than one specimen of faeces been examined from each patient, the proportion of positive results might have been even higher. Whether the same holds true of typhoid fever is not known with certainty, but the observations of A. C. Jones (1953) during the Oswestry outbreak strongly suggest that it does. Using preliminary enrichment in selenite F broth followed by plating on deoxycholate agar, Jones was able to isolate typhoid bacilli from the faeces of 68 per cent. of patients in the 1st week of the disease, of 79 per cent. in the 2nd, of 67 per cent. in the 3rd, of 83 per cent. in the 4th, 80 per cent. in the 5th, and 81 per cent. in the 6th. It is interesting to note, that, according to Glass and Wright (1937), paratyphoid B bacilli are not likely to be isolated from the urine during the first six weeks of the disease unless the faeces are also positive; after this time, however, during the so-called "clearance period," the urine is not infrequently positive when the faeces have become negative. Gell and Knox (1942), in their study of the Kettering outbreak of paratyphoid B fever, reached the same conclusion.

Route of Infection.—We have not yet considered the route by which the bacilli gain access to the tissues and the blood stream during the initial stages of naturally occurring infection. Our knowledge of the epidemiology of enteric fever, and of its mode of spread, makes it quite clear that the bacilli enter the body by

tion, the growth is more like that of the colon bacillus. No great value as yet attaches to the character of growth on potato, for there is wide variation in both the reaction of potatoes and the behavior of different strains of bacilli.

The typhoid bacillus stains easily with the ordinary aniline dyes and is readily decolorized by the Gram method.

Physiology. *Salmonella typhi* is not nutritionally fastidious and grows readily upon the usual nutrient (beef extract) agar and gelatin. These bacteria may be grown on simple synthetic solutions containing glucose and an ammonium salt; some strains appear to require the addition of tryptophane to these solutions,³ but it is probable that this amino acid functions as a growth stimulant rather than an essential food substance.⁴ The typhoid bacillus is a facultative anaerobe, growing almost as luxuriantly under anaerobic conditions

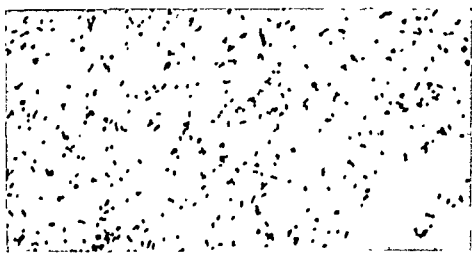


Fig 73. *Salmonella typhi*. Smear from a pure culture, Sommersby strain. Note the variation in size from coccoid to bacillary forms. Fuchsin, $\times 1050$.

as under aerobic conditions. Some growth is apparent at 4°C . and there is no growth beyond 46°C ., while the optimum temperature is 37°C . Like the other intestinal forms, the typhoid bacillus grows over a relatively wide pH range, 5.0 to 8.6, with an optimum at pH 6.8 to 7.0.

Gelatin is not liquefied and indol is not formed, but nitrates are reduced to nitrites. Hydrogen sulfide is produced. As compared to many of the other enteric forms, the typhoid bacillus is a relatively weak fermenter and it resembles *Bact. coli anaerogenes* in that acid and no gas is produced, this last characteristic is of practical value in the glucose fermentation in the differentiation of the typhoid bacillus and *Salmonella* species. The acids produced in the fermentation of glucose are lactic acid for the most part, together with small amounts of ethyl alcohol, formic and acetic acids and, sometimes, succinic acid. Acetylmethylcarbinol is not formed. Although no gas may be observed in the fermentation tube, precise measurement has shown that carbon dioxide is evolved under aerobic conditions, one mol of carbon dioxide being given off

³ Fildes, Gladstone and Knight. *Brit. Jour. Exp. Path.*, 1933, 14:189.

⁴ Burrows. *Jour. Inf. Dis.*, 1939, 64:145, *ibid*, 1939, 65:134.

over long periods of time. Experience with mice (Topley 1926) suggests that the spleen may form a persistent focus of infection; and the well-known typhoid periostitis affords an example of a lesion which may occur long after convalescence from the original illness.

It should be noted that relapses may occur during convalescence, although they are infrequent except in patients treated with antibiotics. They are usually milder and of shorter duration than the original attack; and the fatality rate is low (Gay 1918). They are undoubtedly due to a re-invasion of the blood stream from the tissues in which typhoid bacilli are still proliferating at the time when the bacteremic phase of the primary attack is brought to a close. They are milder, and of shorter duration, presumably because specific antibodies are already present, and because the tissues respond promptly to the secondary stimulus.

Diagnosis

The methods of diagnosis that are available and their relative value at different stages of the disease are indicated by the time-relations shown in Fig. 286. Though at one time reliance was placed chiefly on serological methods of diagnosis—the Widal reaction—modern practice lays far more emphasis on the cultural isolation of the causative organism from the blood, faeces or urine. Serological tests cannot afford more than indirect evidence of infection, and their interpretation may be rendered difficult by past exposure to infection or by previous injection of the patient with T.A.B. vaccine. Moreover, examination of the serum often fails to distinguish between typhoid and paratyphoid fever.

Cultural Methods in Diagnosis.—The method of choice is *blood culture*. This has the great advantage over culture from the faeces, urine or bile of showing not only that the patient is infected with the bacillus, but that the infection is active and is almost certainly responsible for the disease from which he is suffering.

At least 10 ml. of blood should be withdrawn with a syringe or similar device from a vein and transferred to a tube containing an anticoagulant, such as citrate, oxalate, or liquid. On arrival at the laboratory, the blood should be transferred to a flask containing 50–100 ml. of 0.5 per cent. sodium taurocholate broth, or preferably distributed in about 3-ml. quantities into tubes containing 20 ml. of 0.5 per cent. sodium taurocholate broth, tetrathionate broth (Muller 1925), and brilliant green Esbach broth (Ruys 1934). The cultures should be incubated at 37° C. and plated out daily on to MacConkey's agar or some similar medium, and should not be discarded as negative for at least 11 days (Shaw and Mackay 1951). If the only material sent to the laboratory is clotted blood, the serum should be pipetted off, and the clot, after being broken up with a sterile rod, transferred to bile salt broth. A surprisingly high proportion of successful isolations may be secured in this way; and in early cases the clot culture may be positive when the agglutination test is negative (see Soman 1932, 1934, Downie and Fairbrother 1934).

The proportion of positive blood cultures is usually highest in the 1st week of the disease, but, as already noted, in severe and in fatal cases bacteremia may not reach its peak till the 2nd or 3rd week.

As an alternative or supplement to blood culture, an attempt may be made, preferably by sternal puncture, to isolate the organism from the *bone marrow*. Ling, Liu and Chen (1948) recommend this method, partly because the bone marrow is more often infected than the blood and partly because it remains infected for some time after the end of the bacteremic stage.

If possible, cultures from the faeces, and preferably from the urine also, should

for every mol of oxygen taken up.⁵ Other cultural reactions of the typhoid bacillus are summarized elsewhere (p. 416), but, in general, carbohydrate fermentations, with the exception of the failure to ferment lactose, are not of great differential value.

The susceptibility of the typhoid bacillus to deleterious influences is much the same as that of the other enteric bacteria. It is killed by exposure to 55° to 60° C. for thirty minutes and by the usual bactericidal chemicals in a somewhat shorter time than certain more resistant forms such as staphylococci. It persists, rather than multiplies, in nature for a variable length of time. Some bacilli remain viable in ground water for possibly two or three weeks, but in fecal matter in privy vaults and elsewhere they may persist for one to two months.



Fig. 74. Colonies of typhoid bacillus on nutrient agar. Note the characteristic "maple-leaf" irregular margin and slightly roughened glistening surface. $\times 6$.

Toxins. *S. typhi*, like the other enteric bacteria, does not form a soluble toxin, but its cell substance is toxic to the experimental animal upon parenteral inoculation. A polysaccharide-lipid complex which is reported to represent the somatic antigen and endotoxin has been isolated from typhoid bacilli by Boivin and Mesrobian⁶ by trichloroacetic acid extraction (p. 284). A similar substance, but differing in that it contains protein, has been isolated by Morgan and Partridge⁷ by extraction with diethylene glycol and purified by precipitation from aqueous solution with acetone. The contained polysaccharide is immunologically specific, reacting with O antisera, and is probably the same as that studied by Freeman.⁸ An alcohol-soluble substance having toxic properties has been studied by White⁹ and named by him "Q" substance. Similar

⁵ As measured in Warburg respirometers in the writer's laboratory. Carbon dioxide is also given off by aerated broth cultures.

⁶ The work on the typhoid bacillus is discussed by Boivin and Mesrobian. *Rev. d'Immunologie*, 1938, 4:197.

⁷ Morgan and Partridge: *Brit. Jour. Exp. Path.*, 1942, 23:151.

⁸ Freeman. *Biochem. Jour.*, 1942, 36:340.

⁹ White: *Jour. Path. Bact.*, 1932, 35:77, Dennis: *Proc. Soc. Exp. Biol. Med.*, 1939, 42:89.

be obtained, it is advisable to use a combination of one or two enrichment liquid media with two or three selective solid media.' According to Hobbs and Allison (1945), the best two liquid enrichment media for general purposes are sodium selenite and tetrathionate, and the best two solid selective media, for plating either directly or from cultures in enrichment media, are Wilson and Blair's bismuth sulphite agar and Leifson's deoxycholate citrate agar.'

'For the cultivation of urine two or three 10-ml. quantities should be inoculated into liquid enrichment media and plated out after incubation on to the selective media already mentioned. Bile, in smaller quantities, should be treated similarly, and in addition should be plated out directly on to two or three selective media.

Great care should be exercised over the collection of specimens of faeces and urine for examination. In hospitals imperfectly sterilized bedpans and urinals afford a frequent source of contamination, and it is much better to provide every patient from whom a specimen is required with a special grease-proof, cardboard container into which the excreta can be passed directly (see Holt, Vaughan and Wright 1942). Alternatively a rectal swab may be taken, though this is less satisfactory than faeces when the bacilli are scanty (Shaughnessy *et al.* 1948). The faeces should be examined as fresh as possible; if this is impracticable, they should be collected into the buffered glycerol saline solution described by Sachs (1939) or the deoxycholate citrate solution described by Bingxian and Eliot (1940) to prevent the overgrowth of typhoid-paratyphoid bacilli by coliform organisms. Cultures should be made on to two or three of the selective solid media just mentioned, with and without preliminary enrichment. Non-lactose-fermenting colonies may be tentatively identified by slide agglutination (Report 1939), and confirmed by motility, biochemical and tube agglutination reactions (see Bridges and Taylor 1944). It may be noted that the serum of some rabbits may contain agglutinins to the so called α antigen, which is found in certain strains of coliform and *Proteus* bacilli. Non-lactose fermenting colonies, not belonging to the typhoid or paratyphoid groups, may be agglutinated by the serum of such a rabbit, and may give confusing results (Stamp and Stone 1944). The occurrence of the small colony variant of *Salm. typhi* must be remembered (see Morris *et al.* 1943) and also of non-motile strains.

Whatever selective and differential media are employed, the frequency of isolation of typhoid and paratyphoid bacilli will be significantly increased by making repeated platings from the primary enrichment culture. Topley and Fielden (1922) pointed out that, in an ordinary broth culture from a specimen of faeces, various bacterial species succeed one another as the dominant viable organisms, and that it is fairly common to obtain a pure culture of *Bact. coli* on a plate seeded after 24 hours, and a pure culture of *Salm. typhi* on a plate seeded after several days' incubation. In a selective medium, on the other hand, typhoid bacilli tend to develop before *Bact. coli*: thus Waldhecker (1935) notes that plating from a tetrathionate brilliant green bile selective medium after 5 hours' and 10 hours' incubation, instead of after 20 hours' only, considerably increased the percentage of positive results (see also Boecker 1935).

It must, in particular, be emphasized that the isolation of typhoid or paratyphoid bacilli from a specimen of faeces depends more on the proportion of these bacteria to the rest of the enterobacterial flora in the particular specimen examined than on any detail of technique. No reliance can be placed on a single negative result, whatever method of examination is employed; and in carriers or convalescents where the excretion of these organisms may be intermittent, many specimens may have to be examined before a positive result is obtained.

The Agglutination Reaction in Diagnosis.—As we have already pointed out, less attention is now paid than formerly to the agglutination reaction in the diagnosis of enteric fever. There are so many limitations to the test that it should always

substances have been found in quite a number of the enteric bacilli and their relationship to the lipid complexes is not clear. Morgan¹⁰ has studied the pathologic changes produced by the endotoxin in some detail. Intradermal inoculation results in a local edema and erythema followed by necrosis, and intravenous or intracardial injection results in congestion, hemorrhagic extravasation and necrosis in various organs. The liver and bone marrow pathology is very similar to that observed in fatal human cases of typhoid fever. The vascular epithelium is injured also and thrombosis, common in severe human infections, is produced.

Classification and Antigenic Structure. The typhoid bacillus has been given a variety of names, including *Bacillus typhi*, *Bacillus typhosus* and *Bacterium typhosum*. Although closely related to the other enteric bacilli, it was put into the genus *Eberthella* as the type species, first under the name of *Eberthella typhi* and later as *Eberthella typhosa* in the earlier Bergey classifications. The antigenic structure of the typhoid bacillus is given by the formula IX, XII, (Vi):d: and its close immunological relation to the *Salmonellas* is obvious. As a consequence, the English in particular, allocate the it *Salmonella typhi*. The current Bergey's Manual follows this view and now formally classifies the typhoid bacillus as *Salmonella typhosa*. Since the name *Salmonella typhi* has become established to a certain extent, it would seem desirable to retain it.

Vi Antigen.¹¹ In addition to the usual somatic and flagellar antigens, the majority of strains of typhoid bacilli isolated from human infections, some of which are inagglutinable by O antiserum, were found by Felix and Pitt¹² to contain an additional antigen. This was designated the Vi or "virulence" antigen since they believed it was associated with the virulence of the bacteria. This antigen appears to be similar to the somatic antigens, has in fact been reported¹³ to be extracted with trichloroacetic acid, but differs in that it is heat labile in the presence of water. It is, however, relatively heat-stable in absolute alcohol, acetone or glycerin.¹⁴ According to Pijper¹⁵ the type of agglutination produced by the antibody to this antigen differs from the normal H and O agglutination in that a sort of "paresis" is produced in the flagella, resulting in erratic movements and chance contacts between the cells. Though there appears to be no mutual attraction, the bacilli seem to be sticky and, when relatively large areas come in contact, they adhere to form clumps in which the bacilli tend to be arranged side by side.

Practically all strains of typhoid bacilli isolated from cases of the disease contain Vi antigen, though exception has been noted, and more than 95 per cent of strains isolated from carriers contain it. The antigen tends to be lost as the strain is carried on laboratory media and it eventually disappears. A series of steps is apparent in this degradation. The strain is at first inagglutinable by O antiserum and the first step is the acquisition of O agglutinability, often after

¹⁰ Morgan: Amer. Jour. Path., 1943, 19 135.

¹¹ See the review by Almon: Bact. Rev., 1943, 7 43.

¹² Felix and Pitt: Lancet, 1934, ii 186.

¹³ Boivin and Mesrobian: Compt. Rend. Soc. Biol., 1938, 128 5.

¹⁴ Peluffo: Proc. Soc. Exp. Biol. Med., 1941, 48.340.

¹⁵ Pijper: Jour. Path. Bact., 1941, 53 431.

was considerable, but experience has since shown that even the use of standardized agglutinable suspensions does not ensure uniformity of results between different laboratories. In an international experiment described by Gardner (1937), in which 61 sera from cases of typhoid or paratyphoid fever were titrated against standard suspensions, a surprisingly big variation was observed between the results of the four different laboratories that took part. Owing to technical errors and differences of one sort and another, and to the bias of individual workers in reading agglutination end-points, it is frankly impossible to expect different laboratories to obtain identical results. This conclusion must always be borne in mind when interpreting titres recorded by other laboratories. Even in the same laboratory it is always wise, when comparing the agglutinin content of specimens of serum taken from the same patient at different times, to titrate the different specimens simultaneously against the same suspension.

Neither the standard agglutination end-point nor the reduction factor of Dreyer is applicable to O and Vi agglutinable suspensions. Moreover, it is now clear that an antigen cannot at present be used as a standard. The standard must be an agglutinating serum which can be dried and preserved without alteration for a long time (Felix 1950). With such a standard serum a properly prepared suspension should agglutinate to titre. Tests are put up at the same time with the standard serum and the patient's serum, and unless the suspension used agglutinates to the correct titre with the standard serum, it is regarded as unsuitable and must be replaced by a fresh suspension. A provisional International Standard Antityphoid Serum, for both O and Vi antibodies, is available (Report 1953, Felix 1954), and similar standard agglutinating sera for typhoid and paratyphoid A and B fevers are in preparation (Felix and Bensted 1954); and a provisional standard serum for Vi agglutination is issued by the Standards Laboratory for Serological Reagents at Colindale. It is hoped to prepare standard sera against each of the H and O antigens of the typhoid-paratyphoid bacilli.

Before discussing the interpretation of the agglutination test, we must consider three main variables that affect the result.

(1) **Frequency Distribution of Agglutinins in the Population.**—Owing partly to the occurrence of latent and past infections with members of the enteric group of bacilli, partly to previous inoculation with T.A.B. vaccine, and partly to infection with other organisms of the *Salmonella* group or with organisms of the *Bacterium* group possessing antigenic factors similar to those present in typhoid and paratyphoid bacilli, a certain proportion of the population of any country are found to contain antibodies in their serum capable of reacting to a variable titre in the Widal test. Frequency distribution studies of H antibodies were made by Rosher and Fielden (1922) in London, by Smith, McVie and Newbold (1930) in Manchester, by Alves (1936) in Southern Rhodesia, and by Lewin (1934) in South Africa. In addition, Gigholi (1933a) in British Guiana, Gardner and Stubington (1932) in Oxford, Beattie and Elliot (1937) in Edinburgh, and Schwabacher, Ross and Carruthers (1943) in London and Cambridge studied the distribution of O agglutinins. We shall select two series with H and two with O agglutinins to illustrate the type of result obtained.

Table 118 records the findings of Smith, McVie and Newbold (1930) on sera sent in to the Manchester Public Health Laboratory for a Wassermann test during 1925 and 1926. We have extracted the figures for females in order to avoid as far as possible the effect of T.A.B. inoculation. It will be seen that H agglutinins

only one or two transfers. Next the strain loses the ability to stimulate the formation of Vi antibody, then agglutinability with monospecific Vi antiserum disappears, and finally ability to absorb Vi agglutinins from antiserum is lost. Kauffmann¹⁶ has suggested a terminology for these changes which has been generally adopted. A strain inagglutinable in O antiserum is termed a *V strain*, when it agglutinates in O antiserum but retains other Vi characteristics it is a *V-W strain*, and when a strain gives no evidence of the presence of Vi antigen it is designated a *W strain*.

The association of Vi antigen and virulence is not clear-cut, and some workers have expressed doubts of its validity. Virulence is difficult to measure, particularly since typhoid fever is not reproduced in laboratory animals. It may be noted that the same antigen has been found in *S. paratyphi C*, and its presence in these bacteria is not correlated with their virulence (in terms of minimum lethal dose) for mice, their natural host. Nevertheless, Vi antibody has powerful protective properties as assayed by the mouse protection test and appears to be somewhat more efficient in this respect, though qualitatively no different, than O antibody. Antibody to Vi antigen from bacteria other than the typhoid bacillus, such as *S. ballerup*, protects mice against typhoid bacilli as well as antibody to the homologous antigen.

Bacteriophage Typing. It was shown by Craigie and Yen¹⁷ that a number of types of Vi-containing typhoid bacilli may be differentiated on the basis of susceptibility to the lytic action of races of bacteriophage. These types and subtypes are designated by letters, A, B₁, B₂, C, etc., through T to a total of 24 types. There is some cross reaction between subtypes, but very little between types. These phage types are apparently stable. Craigie and Felix¹⁸ have suggested a standardized procedure for phage typing and this or some other should be adopted to assure uniformity in results. Typing is readily accomplished by inoculating a series of areas on an agar plate and, after the inoculated areas have dried, each is inoculated separately with the type phages diluted to act selectively. After incubation an area of lysis is produced by the phage type to which the unknown strain belongs. Phage typing has been of considerable value in epidemiological studies.

Variation. The dissociation of the typhoid bacillus into the usual smooth and rough colonial types is well known. The round, domed, bluish white colonies observed in blood cultures are the typical smooth form, while the rough colonies are flatter, with a roughened surface and irregular edges, and are more opaque. The rough forms are not necessarily non-motile, and in this respect four types may be distinguished: the smooth motile, the smooth non-motile, the rough motile and the rough non-motile. The S-R transformation is, as in the case of the Salmonellas, associated with a change in the immunological specificity of the somatic or O antigens. The Vi antigen is apparently independent and may be present either with or without O antigen. A series of antigenic combinations is, then, possible, for each of the four above types may or may not contain Vi antigen, and H antigen, O antigen and Vi antigen may be present separately or in any combination.

¹⁶ Kauffmann. *Ztschr. f. Hyg. u. Infektionskr.*, 1935, 116 617.

¹⁷ Craigie and Yen. *Canadian Pub. Health Jour.*, 1938, 29 448, 484.

¹⁸ Craigie and Felix. *Lancet*, 1947, 1823.

by Gardner and Stubington (1932) for 50 normal uninoculated persons in Oxford and by Beattie and Elliot (1937) for 47 uninoculated students in Edinburgh. It is apparent that in Great Britain O agglutinins to both typhoid and paratyphoid bacilli tend to occur at a higher titre in normal persons than H agglutinins.

TABLE 121
O AGGLUTINATION: BRITISH GUIANA.

Organism	Percentage of Sera Agglutinating at, or above					
	1/20	1/40	1/80	1/160	1/320	1/640
<i>Salm. typhi</i> O	16.3	3.7	0.9	0.3	0.3	0
<i>Salm. paratyphi B</i> O.	20.3	5.7	2.0	0.3	0.3	0
<i>Salm. paratyphi C</i> O.	19.4	9.4	3.1	2.0	0.9	0.3

British Guiana, however, as is evident from Table 121, the reverse appears to be true. So far as it is justifiable to compare Tables 120 and 121, there is very little difference noticeable, excluding *Salm. paratyphi C*, between the O-agglutinin titre in Great Britain and British Guiana in spite of the great difference in the H-agglutinin titres.

The conclusion that the frequency and concentration of O agglutinins in different parts of the world vary much less than the frequency and concentration of H agglutinins is borne out by other surveys to which we have already referred. It agrees with our knowledge obtained from immunological studies that the antibody response to specific stimulation with O antigens is more transitory than that with H antigens.

(2) Stage of the Disease.—Reference to Fig. 286, p. 1722 and to Figs 263, 264, p. 1278, will indicate that the significance of a positive or negative agglutination test, or of a positive result to any given titre, varies according to the stage of the disease at which the sample of blood is taken.

It is clear that little importance can be attached to a negative agglutination test on a sample of blood taken during the first week of illness. It does not follow that a test made at this time is useless. It is often of value since, taken in conjunction with tests made at a later period, it may enable us to demonstrate that a significant rise in agglutinins has occurred; the demonstration of such a rise has far more diagnostic significance than the demonstration that any particular sample of serum gives agglutination to a particular titre, unless that titre is very high one.

As an illustrative example, bearing in mind our evidence with regard to the distribution of normal agglutinins, let us suppose that we find that a sample of serum taken from a patient in the first week of the disease, gives an H titre of 1/20 against the typhoid bacillus. If the sample had been taken in British Guiana, this finding would have almost no significance, since about 1 normal person in 5 would give this titre. If it had been taken in England it would have been more suggestive; only about 1 normal person in 20 would give this titre. If we found, in addition, an O titre of 1/50, the significance of the H titre of 1/20 would be greatly increased. Similarly, a 1/20 H titre against *Salm. paratyphi C* in England would be very suggestive of infection; in British Guiana it would have almost no significance. If, however, we found these titres in a specimen of serum taken from a patient

The presence or absence of Vi antigen is not associated with the S-R transformation, and the gradual disappearance of this antigen on continued cultivation on laboratory media cannot be regarded as a dissociative change.

The S-R transformation may take place in the body, and it is a common experience to find that typhoid bacilli isolated from carriers are spontaneously agglutinable, avirulent, typically rough forms. The relation of this transformation to the continued presence of the bacilli in the healthy carrier is not clear, though possibly associated with the S-R transformation that may be brought about *in vitro* by cultivation in the presence of immune serum.

Pathogenicity for Man. Typhoid fever (enteric fever, Ger., *Abdominaltyphus* or *typhus*; Fr., *la fièvre typhoïde*) was for long one of the most widespread and important of all bacterial diseases. In the United States in 1900, there were 35,379 reported deaths from this disease, undoubtedly a low figure, and probably some 350,000 cases of typhoid fever in a population of 76,000,000—in the course of a decade perhaps one person in every 20 to 25 contracted the disease. The prevalence of typhoid fever has greatly diminished in recent years, and a large part of this decrease has taken place in the large cities. The total deaths from typhoid fever in 93 cities in the United States with an aggregate population of 38 million were 385 in 1935, 259 in 1939, 95 in 1942, 85 in 1943, 73 in 1944, and 87 in 1945. In 1945 56 of the cities had no deaths, 31 had less than 1 per 100,000 and only 6 a rate of over 1 per 100,000. In the case of 78 cities for which data are available, the rate has fallen from 20.5 in 1910 to 0.2 in 1945.¹⁹ In the country as a whole, 4425 cases and 472 deaths were reported in 44 states in 1945, rates of 3.8 and 0.4 per 100,000 respectively. Typhoid still persists, however, and epidemics occur from time to time, particularly in the smaller towns and rural areas. Much the same situation prevails in other countries, such as Great Britain.

The common symptoms of typhoid fever include frontal headache, lack of appetite, nosebleed, the development of rose spots on the abdomen, muscular weakness and diarrhea. Sometimes considered primarily an intestinal infection, the disease is, in fact, a general invasion of the body, particularly of the lymphatic system. The intestine is often regarded as the main portal of entry of the bacilli into the body, the lymphatic tissues in the intestinal wall are first invaded and the bacilli spread through the lymphatic system. After considerable multiplication has occurred (incubation period), the bacilli overflow into the blood and bacteriolysis takes place; the endotoxins which are liberated as a result of the destruction of the bacilli produce the symptoms of typhoid fever. Other evidence suggests that the body tissues may be invaded through the tonsils and gastric mucosa, but in any case the end result is the same and typhoid fever is a general and not a localized infection.

The typhoid bacillus appears in the blood stream early in the disease, *i.e.*, after the onset of symptoms, and may be cultured within the first ten days in the majority of cases, either from a blood sample or from the clot of samples sent in to a laboratory for agglutination tests. The presence of typhoid bacilli in the blood, however, does not constitute a septicemia; in

¹⁹ Jour. Amer. Med. Assn., 1946, 131:817.

observations on men of the Royal Air Force recorded by Downie and his colleagues (Report 1942c) and reproduced in Table 122 illustrates the high and more persistent titres of H and the low and more transitory titres of O agglutinins after inoculation (see also Beattie and Elliot 1937, J. F. Wilson 1945). As pointed out by Felix (1924b, 1930), the significance of O agglutinins is much greater than that of H agglu-

TABLE 122
PERCENTAGE DISTRIBUTION OF END TITRES AGAINST *Salm. typhi* O AND H AND *Salm. paratyphi* A AND B H SUSPENSIONS.

Time since Last Injection of T A B. Vaccine.	No. of Sera Tested.	Suspension	Per cent. of Sera giving End Titres of					
			<1/100	1/100 <1/200	1/200 <1/400	1/400 <1/800	1/800 <1/1,600	1/1,600 or over
1 week to 3 months	198	TO	95.0	5.0	—	—	—	—
		TH	30.3	21.7	23.8	16.1	6.6	1.5
		AH	5.0	7.6	15.7	30.3	20.8	11.6
		BH	4.0	10.1	20.2	24.8	27.2	13.6
3 months to 1 year	70	TO	97.1	2.9	—	—	—	—
		TH	37.2	25.7	20.0	12.9	4.3	—
		AH	15.7	21.4	12.9	30.0	14.3	5.7
		BH	10.0	10.0	21.4	28.6	18.6	11.4
1 year to 3 years	29	TO	100.0	—	—	—	—	—
		TH	41.4	31.0	13.8	3.8	10.3	—
		AH	34.5	—	20.6	20.6	10.3	13.8
		BH	17.2	20.6	13.8	31.0	10.3	6.9
Total sera 1 week to 3 years	297	TO	96.0	4.0	—	—	—	—
		TH	33.0	23.6	21.9	14.1	6.4	1.0
		AH	10.4	10.1	15.5	29.2	24.2	10.4
		BH	6.7	11.1	19.9	26.2	23.6	12.5

tinins in the diagnosis of enteric fever in the inoculated subject. Nevertheless, even the study of O agglutinins may prove unhelpful or misleading. Dick (1946), for example, experienced a number of false positive reactions in non-enteric cases, and both he and Mole (1948) record the failure of O agglutinins to appear or to rise in titre during the course of genuine enteric fever.

It may be concluded that the only reliable method of diagnosis of enteric fever in the inoculated subject is the isolation of the causative organism.

- *Summarizing*, we may say that agglutinins usually appear in the blood towards the end of the 1st week of illness, increase to a maximum during the 3rd week, and persist for weeks or months after convalescence. The result of the test is thus unlikely to be strongly positive in the 1st week, but is nevertheless of value in that it may be compared with a second test made 5 to 10 days later in a case in which the diagnosis remains doubtful. In the uninoculated subject a rising titre is generally indicative of enteric infection. In the inoculated subject H agglutinins are valueless, but a rise in O agglutinins in a patient inoculated more than 6 months previously is suggestive of active infection, though by no means diagnostic. No one titre can be accepted as certainly significant. In a patient, however, without a history of inoculation with T.A.B. vaccine or of previous enteric infection, living in a country in which the disease is at a very low endemic level, a titre of 1/50 for

fact, probably little or no multiplication takes place. The bacilli are also present in the bone marrow early in the disease, and some have urged culture by sternal puncture to facilitate diagnosis. During and after the second week typhoid bacilli may be found with increasing frequency in the feces, and the proportion of positive blood cultures drops off. The bacilli are also excreted in the urine in perhaps 25 per cent of the cases. They may often be found early in the disease in the rose spots, not in the blood but in the lymphatic spaces.

On autopsy, the intestinal walls are usually found to be extensively ulcerated, Peyer's patches and the solitary glands of the intestine being par-

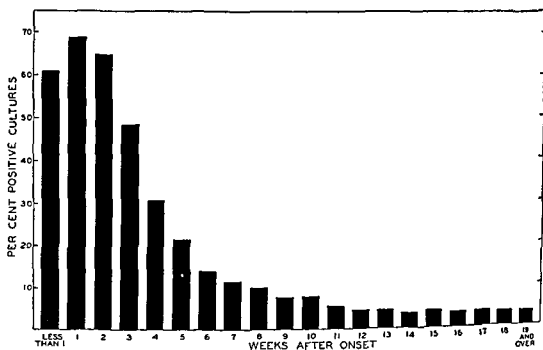


Fig. 75. The persistence of typhoid bacillus infection as indicated by percentage of positive fecal cultures by weeks after onset. Data from 374 cases in New York State exclusive of New York City by Ames and Robins Amer. Jour. Pub. Health., 1943, 33:221.

ticularly involved and containing typhoid bacilli. Perforation of the intestinal wall as a consequence of ulceration is a not uncommon occurrence. The spleen is enlarged and congested and usually contains large numbers of typhoid bacilli. In both the spleen and the liver the bacilli seen in stained sections occur in groups or masses rather sharply focalized; scattered individuals are not often found.

A variety of complications may occur. Laryngeal ulcer is occasionally observed. The gallbladder is not infrequently infected and cystitis sometimes occurs. Suppurative and inflammatory processes may appear in other parts of the body. The osseous system seems especially open to attack, and affections of the periosteum, the bone marrow and the joints have been traced to infection with *S. typhi*. Osteomyelitis may develop as long as six or seven years after recovery from typhoid fever, indicating that the typhoid bacillus can remain in contact with human tissues for years without losing its virulence. Other parts of the body are more rarely invaded during typhoid fever,

reached. As Gay (1918) points out, the incidence in Western countries up to about 1880 was greatest in the cities, where it varied with the density of the population, but after this time the sanitation of the large cities improved rapidly and steadily, with the result that the disease became relatively commoner in small towns and villages which had not made corresponding improvements in the disposal of excreta and the provision of a pure water supply.

TABLE 124

ENTERIC FEVER: ENGLAND AND WALES. DEATH-RATES PER MILLION.

	0-	5-	10-	15-	20-	25-	35-	45-	55-	65-	75 and up
<i>Males:</i>											
1901-10 .	32	62	71	137	179	178	142	104	80	40	17
1921-24 .	3	6	10	20	19	20	17	16	16	13	3
1925 .	6	5		16		14	10	9	11	9	4
1926 .	2	5		11		16	14	15	10	11	7
1927 .	2	8		12		11	12	14	12	5	—
<i>Females:</i>											
1901-10	30	59	81	100	99	97	86	67	50	29	10
1921-24	3	9	11	19	18	16	15	13	7	9	3
1925 .	1	8		12		12	13	11	12	7	7
1926 .	2	5		12		7	12	11	11	4	7
1927 .	4	3		11		9	10	14	12	6	4

The disease used to be a little more frequent in males than females, particularly in the older age groups, but the sex difference was not striking. Since the first world war, however, the sex incidence has been reversed (Chauffard *et al.* 1921, 1922).

As is evident from Table 125, the highest incidence is in adolescence and young adult life, but the case-fatality rate increases throughout life.

TABLE 125

INCIDENCE AND FATALITY OF ENTERIC FEVER IN DIFFERENT AGE GROUPS
(after Godfrey 1928).

Age Group	Incidence (percentage of cases at all ages)	Case Fatality percentage.
Under 5	4.5	8.4
5-9	13.8	4.4
10-14	14.3	6.4
15-19	14.2	10.2
20-24	12.0	12.5
25-29	9.7	13.5
30-34	7.7	16.4
35-39	6.3	15.2
40-44	5.4	15.2
45-49	3.9	19.9
50-54	3.3	22.9
55-59	2.0	22.8
60 and over	2.9	36.5

but almost any organ can be attacked occasionally. The presence of *S. typhi* has been reported in a brain abscess, and the cerebral and meningeal symptoms occurring in many cases of typhoid fever are directly connected with the localization of the bacilli in the meninges—bacilli have been found in spinal fluid obtained by lumbar puncture.

Secondary or mixed infections, especially with the pyogenic cocci and the pneumococcus, are not at all uncommon, and sometimes result in serious complications. Mixed infections with the tubercle bacillus and the anthrax bacillus have also been observed.

Carriers. About one-third of the individuals having typhoid fever discharge bacilli for a period of three weeks after the onset of illness and about 10 per cent for eight to ten weeks; these are known as convalescent carriers. A certain proportion continue to discharge typhoid bacilli for six months or more, and in many cases over a period of several years or throughout the whole of a long life.

The development of the carrier condition is probably dependent upon the invasion of the gallbladder in the case of the fecal carriers and of the urinary bladder in the case of the urinary carriers. Fecal carriers are more common than urinary carriers, and combined fecal and urinary carriers are relatively uncommon. It is not known why women are more commonly carriers than men. In the series studied by Ames and Robins²⁰ 2.1 per cent of the males became chronic carriers as compared with 3.8 per cent of the females. Age is a factor also, according to the same workers the percentage of cases becoming carriers was 0.3 in the 0-9 and 10-19 age groups, but as high as 10.1 in the 50-59 age group. The usual estimates for all age groups vary from 0.5 to 11.6 per cent. Typhoid bacilli need not be excreted continuously; in fact, their intermittent appearance is very common, and weeks may elapse with negative cultures before the bacilli reappear. The necessity for repeated examinations is, of course, obvious. A majority of carriers give the Widal reaction, and in most cases the opsonic index is abnormally high. Antibody to Vi antigen is found in the great majority of carriers, but is only transitory if present at all in inoculated persons, and the use of the Vi agglutination test for the detection of carriers has given encouraging results.

Attempts to cure typhoid carriers by non surgical means, such as chemotherapy, vaccine therapy or bacteriophage, have not been generally successful, and such procedures are not generally advocated at the present time.²¹ Removal of the gallbladder under suitable conditions is often effective in the case of fecal carriers; possibly three-fourths or more are cured.

The proportion of typhoid carriers in the general population is not known, owing to obvious practical and technical difficulties. It is probably quite different in different localities and doubtless depends largely upon the prevalence of current and past typhoid infection. By a modified life table procedure Ames and Robins²⁰ estimated a carrier rate of 42 per 100,000 in New York State. On the basis of reported deaths and assuming a case fatality rate of 10 per cent and a chronic carrier incidence of 2 per cent in

²⁰ Ames and Robins: *Amer. Jour. Pub. Health*, 1943, 33:221.

²¹ See the discussion by Cutting and Robson: *Jour. Amer. Med. Assn.*, 1942, 118:1447; Feemster and Smith: *Amer. Jour. Pub. Health*, 1945, 35:368.

has usually ceased by the time the water supply falls under suspicion. The evidence is, none the less, convincing enough. Water-borne outbreaks are generally characterized by a typically explosive onset; the curve of notifications rises suddenly and steeply, the majority of the cases developing within a relatively few days. The curve for the whole epidemic is often characteristically skew, the primary cases due to direct infection from the water supply being followed by secondary crops of contact cases. Less often, particularly when infection of the water is slight and intermittent, instead of an explosive outburst, there is a series of scattered or "dropping" cases or small groups of cases occurring over a considerable period of time, and affecting only a small proportion of the consumers. Suspicion of the water should always arise if building operations have been proceeding on a gathering ground, or if extensions, repairs or other alterations have been going on in the supply services. The infrequency of water-borne outbreaks of paratyphoid fever has already been noted. One small but clear-cut outbreak in the little village of Brixworth in Northamptonshire, in which a shallow well water was found to be heavily contaminated with paratyphoid B bacilli, was described by Jones, Gell and Knox (1942); and Savage (1942) gives references to two or three other outbreaks suspected of being water-borne. Franklin and Halliday (1937) mention one small outbreak in Maryland of paratyphoid A fever in which a common well was exposed to infection by a person convalescent from the disease. In view of the known survival of paratyphoid bacilli in sewage and the not infrequent access that these organisms must gain to water supplies, their failure, in contrast to typhoid bacilli, to set up disease is most striking. If, however, water containing paratyphoid bacilli gains access to a food in which the organisms can multiply, the disease may follow, as in the milk-borne outbreaks at Wootton in the Isle of Wight (Wallace and Mackenzie 1947) and in South Wales (Thomas *et al* 1948). Sporadic cases of enteric fever may result from bathing in sewage polluted water, but the danger is apparently very slight (see Martin 1947, Stevenson 1953, Steiniger 1953).

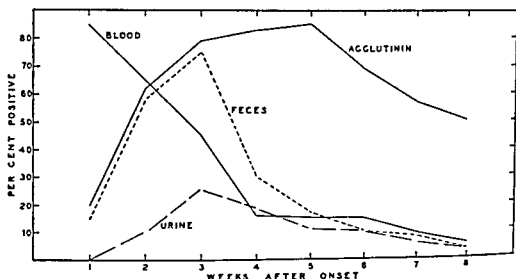
Shell-fish, particularly oysters, are another important source of infection in typhoid fever, though rarely incriminated in paratyphoid fever. They are often bred or fattened in the sewage-polluted waters of tidal estuaries, and they are usually consumed uncooked. Numerous epidemics are on record which can be traced to this source (see Gay 1918), and sporadic cases are likewise common. In France alone Belin (1934) estimated that during the previous fifteen years more than 100,000 cases of typhoid fever had occurred due to the consumption of shell fish, of which 25,000 had ended fatally. The relation between the consumption of shell-fish and the incidence of typhoid fever in a large city was considered in some detail by Niven (1910) in a study of the seasonal prevalence of typhoid fever in Manchester.

Milk follows close on water as an important source of sudden and massive herd infection. Ballard, in pre-bacteriological days, adduced good circumstantial evidence for inculcating a polluted milk supply in a localized epidemic at Islington. Schuder (1901), who studied the records of 640 epidemics, attributed 110 to contaminated milk.

In this country there are numerous records of milk-borne outbreaks of typhoid and paratyphoid fever, of which the 1931 outbreak of paratyphoid fever at Epping affecting 260 persons and causing 8 deaths (Bullough 1931), and the 1936 outbreak of typhoid fever at Bournemouth, Poole and Christchurch affecting at least 718 persons and causing

survivors, Anderson, Hamblen and Smith²² estimated a carrier rate of 48 per 100,000 in Massachusetts, and Grey²³ similarly estimated a rate of 288 in Mississippi. The number of carriers throughout the United States is probably decreasing since they are no longer produced in such quantities as when typhoid fever was prevalent. By extrapolation Ames and Robins²⁰ calculated that the 2500 carriers in New York State in 1940 would be reduced to 200 by 1980.

Bacteriological Diagnosis of Typhoid Bacillus Infection. The isolation and identification of the typhoid bacillus is essential in the detection of the carrier state and is necessary to establish a diagnosis of typhoid fever. The isolation of the bacillus from blood or urine specimens is ordinarily a relatively simple matter since the fresh specimen is not heavily con-



The approximate incidence of positive culture of blood, feces and urine, and agglutinin response in typhoid fever.

taminated. Fecal specimens, however, contain very large numbers of *Bact. coli* and other bacteria and selective as well as differential media are required.

The procedure is essentially the same as that used for the isolation of other *Salmonella*, and includes enrichment culture in Selenite-F broth and direct plating on S-S and D-C agars and on MacConkey agar. Blood is usually cultured in bile broth or brilliant green bile broth, though cultures in nutrient broth are often satisfactory. The bismuth-sulfite agar of Wilson and Blair is particularly useful for the isolation of the typhoid bacillus for the colonies on this medium are black and distinctive; however, *S. paratyphi* B and *S. enteritidis* also grow as black colonies. The medium is strongly inhibitory and must be inoculated very heavily; consequently, colonies may not be pure cultures. Growth on this medium is often not a satisfactory agglutinating antigen. The medium is difficult to prepare in uniform quality but because of its utility is very widely used.

The typhoid bacillus is identified by biochemical reactions and specific

²² Anderson, Hamblen and Smith: Amer. Jour. Pub. Health, 1936, 26 396.

²³ Grey: Amer. Jour. Pub. Health, 1938, 28:415.

remission before the onset of enteric symptoms, or there may be complete and lasting recovery.

Thus, in the Consett paratyphoid outbreak in 1940, which was due to home-made trifle infected from a profuse faecal and urinary excreter who was probably in the convalescent stage of an ambulant infection, of 56 patients admitted to hospital, 7 contracted enteric fever after an incubation period of six to twelve days; 17 suffered from gastro-enteritis coming on 12-72 hours after consumption of the trifle and gradually passed into the enteric state; 17 suffered from gastro-enteritis, recovered more or less completely, and then developed enteric fever after an interval of a few days; 9 suffered from gastro-enteritis and recovered completely; and 6 suffered from no symptoms at all but were proved bacteriologically to be infected (Warren 1941).

Whether the initial gastro-enteritis represents the first reaction of the tissues to the specific infecting organisms, or whether it is due to other organisms capable of causing food poisoning, or to pre-formed toxins, is still in doubt.

'Human hands are probably the principal agents in the conveyance of typhoid or paratyphoid bacilli from faeces to food; but there is another agent, the fly, which may be of considerable importance as a carrier when allowed access to infected excreta.' Its activities in this direction are particularly dangerous in tropical countries, and under the imperfect sanitary conditions which often prevail among armies on active service. Reed, Vaughan and Shakespeare (1899) drew attention to the clear association between the prevalence of typhoid fever among the American troops in the Spanish-American War, and the exposure of excreta; and suggested that flies played an important part in the spread of the disease.

The mechanism concerned in the carriage of *Salm. typhi* by flies was studied experimentally by Firth and Horrocks (1902), Ficker (1903) and Graham-Smith (1910). 'The danger of contamination from the soiled feet, or proboscis, appears to be of relatively short duration; but the observations of Ficker and of Graham-Smith suggest that a far more important source of infection is provided by the fly which itself becomes infected, and carries the bacilli for some days in its intestinal canal.' The observations of Fauchnie (1909), who examined flies caught in infected areas, and isolated typhoid bacilli on several occasions from the crushed bodies of the insects after the exterior had been sterilized by flaming, point in the same direction (see also de la Paz 1939). Paratyphoid bacilli are said to survive for 3-4 weeks in the bodies of mosquitoes and to be excreted in the faeces, but the danger of contracting enteric fever from a mosquito bite seems to be very slight (Braun and Caspari 1939).

The Typhoid and Paratyphoid Carrier.—As has been indicated above, the ultimate source of enteric infection would appear, in all cases, to be the excreta of an infected human being, and this source is seldom remote. 'There is little evidence that typhoid or paratyphoid bacilli survive for any length of time outside the human body under natural conditions.' The mode of survival of the bacilli throughout non-epidemic times, and the starting-point of outbreaks in localities which had long been free from the disease, remained in doubt until the early years of the present century, when the problem was solved during the intensive investigation undertaken in S.W. Germany under the auspices of Robert Koch. Horton-Smith (1900) had previously recorded the case of a urinary carrier of *Salm. typhi*, but the full significance of the chronic carrier was not appreciated until the results of the German campaign were reported. (For an excellent description and discussion of these results see Ledingham and Arkwright 1912.) Koch's thesis, that

agglutination and must be differentiated from other enteric bacilli which cause clinically similar disease.

Epidemiology. The typhoid bacillus is a strict parasite found only in man. Outside the human body multiplication, if it occurs at all, is insignificant, and for practical purposes may be neglected as a factor in the dissemination of the disease. As indicated above, the typhoid bacillus leaves the body in the feces or, less commonly, the urine, and enters the body of a new host via the alimentary tract. The epidemiology of typhoid fever, then, is predicated upon the connection between the intestinal tract of the infected person and the mouth of the susceptible, and the factors that determine the spread of this disease are, essentially, those arising as a consequence of the interrelationships of the individuals or groups of individuals comprising the host population. The extent of the spread of typhoid is, of course, dependent upon the nature of the connecting links between individuals, and two epidemiological types of the disease may be distinguished, the one epidemic typhoid, and the other endemic, or residual, typhoid.

Epidemic Typhoid Fever. Extensive outbreaks of typhoid fever necessarily involve a connecting link that is common to a great many people, and by far the most important vectors of this kind are water and milk. As pointed out elsewhere (Chapter 10), water-borne typhoid fever, formerly all too common but by now relatively rare in the larger communities, arises as a consequence of the contamination of a water supply with infectious fecal material, either as such or in the form of sewage. Water-borne epidemics of typhoid fever occur in the absence of chlorination, filtration and other purification procedures and may, of course, be readily prevented. These epidemics tend to occur in the cold months of the year, particularly in the winter and early spring, and the incidence of the disease is unaffected by age, sex or economic status.

Milk-borne typhoid fever, at the beginning of the twentieth century second only to water-borne typhoid in extent and importance, follows the route of the milkman and, as might be expected, tends to occur in the lower age groups and in families of higher economic status. The general introduction of the process of pasteurization has practically eliminated milk-borne typhoid fever from the larger urban centers, but epidemics continue to occur from time to time in various parts of the country.

Food-borne typhoid fever may take on epidemic proportions in certain instances. Oysters and other shellfish have come into bad repute in this respect in recent years, for a number of typhoid epidemics in Great Britain and the United States have been found to be due to the eating of oysters grown near sewer outfalls or placed to "fatten" in the polluted waters of estuaries or creeks. Watercress, lettuce, radishes or any vegetables or fruits which are liable to come in contact with contaminated water or are sprayed with human excrement may give rise to small-scale epidemic typhoid fever.

Endemic Typhoid Fever. Although epidemic typhoid fever is largely eliminated in a given community through adequate sanitary control of water and milk supplies and such food supplies as are susceptible to the application of effective control measures, the disease remains in an endemic form which is manifested as occasional cases or small groups of cases which

proportion of permanent carriers of typhoid bacilli. Our knowledge, however, is still very deficient, and all our conclusions must be expressed with caution. We may deal first with the *typhoid carrier*.

Lentz (1905) found that 4.5 per cent. of 400 typhoid convalescents excreted *Salm. typhi* for more than 10 weeks, and 3 per cent. for longer than 13 months. Brückner (1910) found 12 carriers among 316 persons who had suffered from typhoid fever in previous years. Kayser (1907) carried out a re-examination of specimens of urine and faeces from 101 persons who had passed through an attack of typhoid fever at least one year earlier, and had been discharged as free from bacilli; 3 of these were found to be again excreting the infecting organism. Gill (1927) refers to the finding in Alabama of 9.5 per cent. of chronic typhoid carriers among a group of 348 persons who had suffered from typhoid fever or from continued fever of several weeks' duration more than a year previously. Gray (1938) in Mississippi found that of 244 proved cases of typhoid fever, 8 continued to excrete the bacilli for over a year; it is to be noted that several patients excreted the bacilli for 3 to 9 months after recovery, but ceased to do so after 12 months. Ames and Robins (1943) in New York State refer to 90 chronic carriers resulting from 3,130 typhoid patients notified during the years 1930 to 1939. Morzycki (1949) in Poland, who examined 10,402 typhoid convalescents, found that 1.3 per cent. of males and 2 per cent. of females were still excreting the organism two years after the attack.

From these figures it is fairly clear that about 3 per cent. of recognized clinical cases of typhoid fever become permanent carriers, though the proportion may in some places be higher.

Klinger in 1909 summarizing the results obtained to that date, gives records of 431 carriers; 211 of these did not excrete the bacilli for more than three months; 220 excreted them for a longer time. The distribution of these carriers, according to sex, and according to the presence or absence of a history of typhoid fever, was as follows:

	Males	Females
Carriers of less than 3 months' duration		
(a) History of attack	31	61
(b) No history of attack	58	61
Carriers of more than 3 months' duration		
(a) History of attack	33	143
(b) No history of attack	5	39

Two points of interest emerge from this distribution: (a) the high proportion of carriers in the first group in which there was no history of a previous attack, and (b) the striking preponderance of females among the second group of carriers. The difference between the sexes is of the same order as that noted for the occurrence of gall-stones. Klinger's figures also show that transitory carriers occur mainly in children and young adults, chronic carriers among those in middle or later life.

Our information on the frequency of typhoid carriers among the population at large is somewhat scanty.

clinical symptoms, either before or after the isolation of the bacilli were found once only. In 2 they were found 3 times; but in no case did they persist longer than 14 days. The other 4 carriers were of the chronic type. Two had passed through an attack of typhoid fever in previous years; from the remaining 2 no history could be obtained. Welch, Dehler and Havens (1925) report the discovery of 39 carriers of *Salm. typhi* during the examination of 1,076 persons engaged in the milk

appear from time to time. The seasonal incidence is quite different from that of water-borne typhoid; the marked increase in incidence in late summer and early fall is not explained (Fig. 76). The source of infection is, of course, the case, frank or ambulatory, or the healthy carrier. Instances of direct, contact infection are unquestionably more common than is generally recognized, and the dissemination of typhoid bacilli from the infected individual to his immediate associates is undoubtedly responsible for the majority of cases of residual typhoid. Carriers are, of course, of particular importance in this connection in that they constitute semipermanent foci

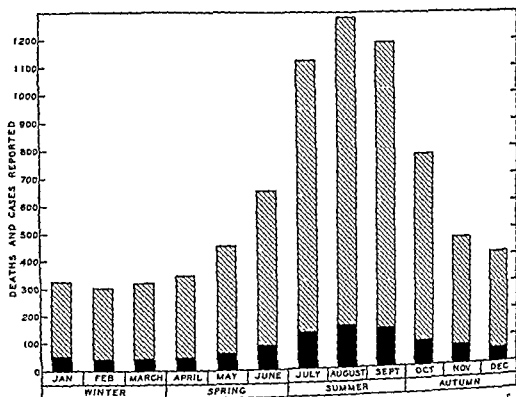


Fig. 76. The seasonal incidence of typhoid and paratyphoid fever. Averages of reported cases by months for the years 1939 to 1945 inclusive. Data from Supplements to Public Health Reports.

of infection and, when employed as food handlers, may be the cause of small epidemics. The most notorious instance of this kind was that of Mary Mallon, "Typhoid Mary," who was unknowingly the cause of some twenty-six cases of typhoid fever in seven different families. Under special circumstances, such as prevail among troops, contact infection may assume epidemic proportions.

The reduction in the prevalence of typhoid fever in the last few decades (Fig. 77) is attributable almost entirely to elimination of the great water-borne and milk-borne epidemics. As noted above, epidemics still occur, and their control is a matter of putting into practice existing knowledge. Residual typhoid, however, is much more difficult to control; the detection and supervision of all carriers, or even the elimination of carriers as food handlers, is a practical impossibility. There is reason to believe that with continued control of epidemic typhoid fever, the reduction in the proportion

proportion of permanent carriers of typhoid bacilli. Our knowledge, however, is still very deficient, and all our conclusions must be expressed with caution. We may deal first with the *typhoid carrier*.

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Our information on the frequency of typhoid carriers among the population at large is somewhat scanty.

Klinger (1906) examined the excreta of 1,700 persons, living in an area in which typhoid fever was endemic, and isolated *Salm. typhi* in 15 cases. Eleven of these presented no clinical symptoms, either before or after the isolation of the bacilli. In 9 cases the bacilli were found once only. In 2 they were found 3 times; but in no case did they persist longer than 14 days. The other 4 carriers were of the chronic type. Two had passed through an attack of typhoid fever in previous years; from the remaining 2 no history could be obtained. Welch, Dehler and Havens (1925) report the discovery of 39 carriers of *Salm. typhi* during the examination of 1,076 persons engaged in the milk

of carriers may well be reflected in a reduced incidence of the disease in the endemic form.

Pathogenicity for Lower Animals. The injection of typhoid bacilli into experimental animals produces much the same effect as the injection of colon bacilli. When introduced into the peritoneal cavity in considerable quantity many strains produce symptoms of a non-specific character and a fatal outcome. Although a genuine but slight multiplication of the bacilli takes place and attests the occurrence of a true infection, neither the symptoms nor the lesions of this intraperitoneal typhoid bear any close resem-

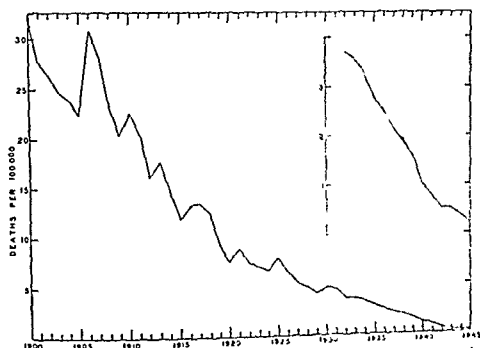


Fig. 77. The prevalence of typhoid and paratyphoid fever in the Peruvian Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

blance to the typhoidal processes in man. The virulence of the typhoid bacillus for mice on intraperitoneal inoculation is fixed to a very high level, only a few hundred sufficing to produce a fatal infection by suspension in 5 per cent mucin (see Army studies below). Berding, et al.,³¹ however, was able to set up an infection by feeding vegetables smeared with typhoid bacilli to fasting rabbits and rats, but without the reproduction of the clinical picture of human typhoid. Others have reported the reproduction of characteristic typhoid lesions in the duodenum in chimpanzees fed typhoid cultures in milk and broth. It may be noted that the enteric state may be produced in the rabbit by intravenous inoculation, the bacilli localizing in the gallbladder, where they may persist for several months. It must be admitted, however, that typhoid fever as it exists in man has never been reproduced in the experimental animal.

³¹ Berding, *Ann. Inst. Pasteur*, 1897, 11: 824.

were still excreting the bacillus after six months. In an outbreak at Liverpool, Glass and Wright (1937) found that the rate of clearance of paratyphoid bacilli from the faeces was most rapid during the second month; about 4 per cent. of convalescents were still excreting the organisms after 15 weeks. In a later outbreak at Liverpool, described by Holt, Vaughan and Wright (1942), the proportion of patients who continued to excrete paratyphoid bacilli after 16 weeks was 5 per cent. The rate of clearance, however, may be much more rapid. In the Bristol outbreak of 1940, for example, described by Davies, Cooper, Wiseman and Davies (1940) the proportion of carriers had fallen to 6.9 per cent. after six weeks, as opposed to figures of 67 and 44 per cent. respectively for the two Liverpool outbreaks just quoted. In the Kettering outbreak described by Gell and Knox (1942), 3.6 per cent. of the patients were still excreting paratyphoid B bacilli after seven months, and in the Jarrow-Hebburn outbreak described by Kennedy and Payne (1950) 1.4 per cent. after five months; for 501 paratyphoid convalescents examined by Vogelsang and Boe (1948) at Bergen, the corresponding figure was 3.6 per cent. after three months. These figures are not all strictly comparable owing to the different methods of estimating the clearance rate. As pointed out in a valuable article by George, Harvey and Scott Thomson (1953), the clearance rate should be based not on the total number of cases, but on the number known to be positive at the week under consideration.

The proportion of chronic carriers of paratyphoid B bacilli is still a matter of conjecture. Gill (1927) mentions that among 348 persons who gave a history of typhoid fever or of continued fever a year or more previously, 12 carriers of paratyphoid A or B bacilli were found. There is reason to believe that most of these were paratyphoid A carriers, but the exact figures are not given. All we can say at present is that chronic carriers of paratyphoid B bacilli do occur, but that their frequency is unknown.

There is little exact information on the duration of excretion of paratyphoid A bacilli, but the observations of Bumke (1926) suggest that these organisms disappear from the faeces at a considerably greater rate than typhoid bacilli.

The literature contains numerous and well-authenticated records of the danger of the chronic carrier. For accounts of such classical instances as the case of the Strassburg Master-Baker's Wife, the case of the Folkestone Milker, or the case of "Typhoid Mary," the student is referred to the excellent monograph of Ledingham and Arkwright (1912), and to a paper by Soper (1939). It need only be noted that in these, as in almost all subsequent cases, the carrier who has achieved publicity has been in some way concerned with the handling of food, and that a large proportion of milk-borne epidemics, or small outbreaks due to the consumption of such food-materials as ice-cream, have been traced to a carrier who has been concerned in their handling or transport.

Laboratory Detection of the Carrier.—In searching for a typhoid carrier among a given sample of the population, much help will be obtained by testing the serum of all suspects for agglutinins before proceeding to the cultural examination of faeces and urine. A carrier often shows the presence of H or O agglutinins, even though only in low titre; but the H agglutinins will afford no useful information if the person has been inoculated with T A B. The observations of Felix (1938a) showed that much greater value attaches to the demonstration of Vi agglutinins. In chronic typhoid carriers the frequency of a positive Vi reaction in the serum appears to be very high, though in transitory carriers Vi agglutinins are usually present only in low titre or may be absent. Felix's observations were confirmed by Eliot (1940), Eliot and Cameron (1941), Klein (1943) and several other workers (see Pijper and Crocker 1943). It is clear, however, that though probably over

Immunity. An attack of typhoid fever confers a certain degree of immunity, although instances of two or even more attacks in the same individual are not unknown. Animal experiment has shown that it is possible to obtain a high degree of immunity in rabbits and guinea pigs against intraperitoneal inoculation. The immunity is associated with the development of humoral antibodies such as agglutinins, precipitins and the like. Lysin is also produced, and, like the cholera vibrio, the typhoid bacillus undergoes visible dissolution and disintegration in the peritoneal cavity of the immune animal.

In man recovery from typhoid fever is also accompanied by the appearance of demonstrable antibodies. In most instances agglutinins appear during the course of the disease, sometimes as early as the fifth day (over 90 per cent by the fourth week), and their presence is the basis of the Widal test used for diagnostic purposes.

In its original form the Widal test was a slide agglutination test, and agglutination of typhoid bacilli by patient's serum in a dilution of 1:50 or more was considered positive. The development of knowledge of the antigenic structure of the typhoid and paratyphoid bacilli in recent years has resulted in a somewhat better understanding of the value and limitations of the agglutination test in the diagnosis of typhoid fever. At the present time the test is a macroscopic one and is carried out with both *H* and *O* antigens. The interpretation of a single such test must take into consideration ancillary data such as a previous immunization or attack of typhoid fever, the prevalence of endemic typhoid in the general population, etc. It is, therefore, difficult to set arbitrary limits, in most instances an *O* titer of 1:100 and an *H* titer of 1:200 may be regarded as significant. A point of some interest is the lack of sharp antigenic specificity of human sera as compared with the specificity of experimentally produced rabbit antisera.

The interpretation of the Widal test in immunized persons is often difficult, since both *H* and *O* agglutinins are formed in response to the vaccine. Titers fall after immunization, of course, but may persist at moderate levels for many months. Furthermore, the agglutinin titer may rise in anamnestic response to a febrile condition. The extent to which this occurs is not definitely known and some data suggest that it is significant while others do not. Such an anamnestic reaction is particularly prone to occur in typhus fever, and typhoid agglutinin titers as high as 1:800 have been observed.²³ There is some evidence that an "agglutinin curve," obtained by periodic agglutinin titrations, has diagnostic value in that it continues to rise in typhoid fever but usually does not do so in the anamnestic reaction.

Prophylactic Inoculation. Man may be actively immunized against typhoid fever by the parenteral inoculation of killed typhoid bacilli. Anti-typhoid vaccination has had its widest application thus far in the protection of soldiers in the field, for, owing to the conditions under which they must live in the field or on the march, the likelihood of typhoid fever is great. Mass immunization has effected a remarkable reduction in the incidence of typhoid fever among these men. For example, in 1898 at the time of the Spanish-American War, 4422 cases of typhoid and 248 deaths occurred in a division of 10,759 men; among 12,801 vaccinated men under very similar

²³ Supplc: Arch. f. Hyg. u. Bakt., 1943, 129:158.

tributary sewers, and thence to the drains from individual houses, it is often possible to demonstrate the actual house in which the carrier is living (see also London and Mackenzie 1951, Moore *et al.* 1952).

The epidemiological significance of the carrier state, whether transient or permanent, is much enhanced by making use of the *bacteriophage method of typing*, employing the scheme devised by Craigie and Felix (1947) for typhoid bacilli and by Felix and Callow (1951) for paratyphoid B bacilli. As this subject has already been discussed in Chapter 30, we need do no more here than remind the reader that the susceptibility of a given strain to lysis by the bacteriophage is intimately associated with the Vi antigen, and that the different bacteriophage types are remarkably specific. For the value of the method in epidemiological investigations reference may be made to papers by Cruickshank (1947), Martin (1947), Felix (1951a) and Felix and Anderson (1951b) in this country, Crocker (1947) in South Africa, Desranleau (1947) in Canada, Henderson and Ferguson (1949) in the United States, and Joe (1949) in Indonesia, and for a review of the carrier problem in general to the monographs by Ledingham and Arkwright (1912) and Browning and his colleagues (1933).

Prevention of Enteric Fever.

General Measures.—Typhoid and paratyphoid fever are specifically human diseases, and the ultimate source of infection lies in the human patient or carrier. As in other infectious diseases, the general problem of prevention consists in eradicating the source of infection, and in blocking the various routes by which the organism may gain access to the body.

Eradication of infection at the source implies successful treatment of the patient and prevention or cure of the carrier state. Treatment with chloramphenicol, which is referred to later, is helpful during the acute stage of the disease, but is of little or no use for the chronic carrier. Experience, however, has shown that a high proportion of both typhoid and paratyphoid carriers can be freed from their infection by cholecystectomy (see Browning *et al.* 1933, Vogelsang and Bøe 1948). Since persons who have been excreting the bacilli for over a year seldom clear up spontaneously (but see Littman *et al.* 1948), this operation should be seriously contemplated for all chronic carriers. The bacilli may be found in the faeces for a time after the operation, but eventually disappear (see Littman *et al.* 1949). In the occasional carrier, however, even removal of the gall-bladder proves a failure; it is not yet clear in what part of the intestinal tract the focus of infection persists, though there is reason to believe that it is the biliary passages of the liver. Urinary carriers present much less of a problem, the urine can usually be sterilized by appropriate antibacterial agents with resultant permanent cure.

Apart from curing the chronic carrier, much may be done to recognize the carrier state and to institute suitable measures of control. The most practicable method of doing this is to follow up each convalescent patient.

Before discharge from hospital the patient should have his blood examined for Vi agglutinins; these will normally be found in about half the convalescent cases. If they are absent in a titre of 1/5, nothing further need be done; the patient may still be excreting typhoid bacilli in the faeces or urine, but is unlikely to continue to do so for long. If, on the other hand, Vi agglutinins are present, a second serum examination should be made three months later. Disappearance of the Vi agglutinins or a considerable fall in titre at this stage may be regarded as satisfactory. Persistence of the original titre,

conditions during summer maneuvers at San Antonio, Texas, in 1911, only one case developed. Similar results have been obtained in the armies of other nations, and the efficacy of typhoid immunization is undoubted. Typhoid vaccination has been compulsory in the United States Army since 1911, and the result has been the practical disappearance of the disease.²⁶ The immunity so developed is not absolute, of course, and may be broken down by large doses of typhoid bacilli, typhoid fever in immunized personnel of armies is observed from time to time.²⁷

The vaccine ordinarily consists of a saline suspension of killed bacteria. The microorganisms are grown on the surface of agar culture media and after eighteen hours' incubation are washed off with sterile physiological salt solution and killed by heating to 55° to 56° C. for one hour. The suspension is standardized by counting the number of bacteria and then diluted

first consists of 0.5 ml. or 500 million bacilli, and the second and third 1 ml. or 1 billion bacilli each. As a rule, the reaction following inoculation is not severe, although fever, chills, nausea and nervous symptoms may be observed. At times it has been found advantageous to immunize simultaneously against the paratyphoid infections as well. Such vaccines, known as TAB vaccines (typhoid, para A and para B), are standardized to contain 1 billion typhoid bacilli, 250 million paratyphoid A bacilli and 250 million paratyphoid B bacilli per ml. Under exceptional circumstances still other microorganisms have been added to typhoid vaccines.

While the effective immunogenic potency of typhoid vaccines can, in the last analysis, be measured only by field trial, protection against the experimental infection of mice with bacilli suspended in mucin has been useful. The active and passive mouse protection tests have been used by workers in the United States Army Medical School in extensive studies directed toward improvement of the immunizing antigen.²⁸ It is reasonably well established that antibody to the O antigen is protective while that to the H antigen is not, and it is also clear that antibody to Vi antigen is protective against infection with Vi-containing bacteria. In general, then, the vaccine should contain adequate amounts of undenatured O antigen and its immunizing potency may also be reinforced by the presence of Vi antigen since most strains of typhoid bacilli isolated from human infections contain it. The Army investigations have shown that highly immunogenic strains of the typhoid bacillus are highly virulent and conclude that such strains should be used for vaccine preparation. The classic Rawlings strain was found to be inferior as assayed by mouse protection and a highly virulent, Vi-containing strain, No. 58, has been substituted for it in Army vaccine. Subsequent

²⁶ The Army experience to 1942 with prophylactic inoculation has been summarized by Callender and Luippold. *Jour. Amer. Med. Assn.*, 1943, 123:319.

²⁷ For experience in World War II see, for example, Jordan and Jones. *Lancet*, 1945, ii, 1007.

troops stationed abroad. These figures were analysed in a valuable paper by Greenwood and Yule (1915), to which reference should be made by all those who are interested in the general question of interpreting statistical evidence of this kind. Employing the method of the fourfold table, and including as inoculated all those who had received typhoid vaccine at the time of the last available return, the results are set out in Table 127.

TABLE 127

INCIDENCE OF TYPHOID FEVER IN INOCULATED AND UNINOCULATED PERSONS.

	Not Attacked.	Attacked.	Total.
Inoculated. . .	10,322	56	10,378
Not inoculated .	8,664	272	8,936
Total	18,986	328	19,314

$$\chi^2 = 180.33$$

$$P = \text{less than } 0.0001.$$

The value χ^2 is a measure, devised by Pearson, of the probability that the distribution actually observed might have arisen as the result of chance; as χ^2 increases, this probability decreases. The value P gives this probability in the usual numerical form. In this particular case the odds are greater than 9,999 to 1—actually they are much greater—against the observed distribution having arisen as the result of chance. Re-examination, however, of this report renders it very doubtful whether these results can be taken at their face value (Cockburn 1953). The method of carrying out the investigation was not such as would be approved now with our greater knowledge of the inherent fallacies of field trials. For example, the vaccinated group consisted of volunteers; inoculation was often carried out during or after an outbreak of enteric fever in the unit; the duration of exposure of the vaccinated and uninoculated groups was not recorded and was almost certainly different; and the diagnosis between typhoid and paratyphoid fever was not always clearly established.

War conditions provide an admirable breeding ground for enteric fever; and it might have been expected that the war of 1914–18 would have yielded a final proof of the efficacy of antityphoid inoculation and a close measure of its exact protective value. There was in fact a great reduction in the incidence of typhoid fever on that observed in the Boer War, the incidence being only 2.35 cases per year per 1,000 strength as compared with 105 previously (see Harvey 1929). But the conditions prevailing in the two wars were very different and the records were not altogether satisfactory. Moreover, as Spooner (1953) pointed out to us, between the two wars the Army adopted chlorination of water. Direct comparison, therefore, of the incidence of typhoid fever between 1899–1902 and 1914–1918 is unprofitable. On the other hand, comparison of the incidence of typhoid and paratyphoid fever in the first world war is worth noting. During the period in which antityphoid inoculation alone was being practised the morbidity from typhoid fever among the inoculated was 0.95 per 1,000 and among the uninoculated 10.35 per 1,000. The corresponding figures for paratyphoid fever were 21.5 and 39.8 per 1,000. If the records are reliable, it looks as if antityphoid inoculation afforded some protection against paratyphoid fever, which in view of the partial sharing of the O antigens of the typhoid and paratyphoid B bacillus is not surprising. The fact, however, that the incidence of typhoid fever was reduced nearly 11-fold in the inoculated compared with a reduction in paratyphoid fever of less than twofold may perhaps be interpreted in favour of the protective value of inoculation.

There is evidence, as might reasonably be expected, that the decrease in morbidity from typhoid fever was associated with a lowering of the case-fatality rate. Among 1,728 inoculated patients in the British armies in France this was 4.57 per cent. as against

work²⁹ has suggested that the vaccine may be further improved by fortification with Vi-containing extracts but such possibilities are still in an experimental stage. Presumably results based on mouse assay may be applied to man but as yet there is no definite evidence that vaccine prepared with strain No. 58 confers a more effective prophylactic immunity in man than Rawlings strain vaccines.

At the present time typhoid vaccines prepared in the United States must conform to an immunogenic potency standard based on the active mouse protection test. "Each of 30 or more mice of any susceptible strain, 6-8 weeks old and weighing 14-16 gms., is given 0.5 ml. of a 1:10 dilution of the vaccine intraperitoneally. Equal numbers of male and female mice should be used in each group. Fourteen days after the injection of vaccine the mice are divided into three groups of not less than 10 mice each, one group to receive

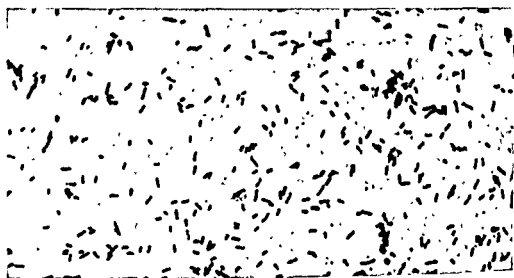


Fig. 78. *Alcaligenes fecalis*. Smear from a pure culture. Fuchsin; $\times 1050$.

approximately 100,000, one group approximately 10,000 and the third group approximately 1,000 lethal doses of virulent typhoid bacilli (16-20 hours old) suspended in 5 per cent mucin." At least 50 per cent of the mice should be protected against not less than 10,000 lethal doses of organisms.

Another type of antigen, consisting of a formalized solution of the cell substance of typhoid bacilli and designated endotoxoid vaccine, has been used in recent years with encouraging results,³⁰ but it is not as yet clear how this antigen compares with whole bacilli.

As indicated above, typhoid vaccines are ordinarily administered parenterally, by subcutaneous injection. It has been contended by some workers that oral administration of the vaccine together with a small quantity of bile, which presumably increases the permeability of the intestinal wall, produces an equally effective immunity. As pointed out in an earlier chapter, oral vaccination is a highly uncertain method of inoculation having no

²⁹ Luippold: Amer. Jour. Pub. Health, 1946, 36:15.

³⁰ Grasset. Brit. Med. Jour., 1939, ii:58; *ibid.*, Pub. South African Inst. Med. Res., 1945, 9:163. See also Morgan, Favorite and Horneff. Jour. Immunol., 1943, 46:301.

1953) confirmed Felix's contention that it is superior to the phenolized product. In the original dosage it gave rise to rather less local, and considerably less constitutional reaction than the phenolized vaccine (Climie 1942, Report 1942b, Drysdale 1947). After the war, however, the strength of the vaccine was doubled, and no difference was then observable in the reactions caused by the two vaccines.

The preparation, standardization and testing of typhoid vaccine require great care; otherwise erroneous conclusions on its value may easily be reached. We have insufficient space to consider this subject here, and would refer the reader to the paper by Felix (1951b) for a description and critical discussion of the numerous technical processes concerned. Suffice it to point out firstly that suitable strains must be selected having not only a high content of Vi and O antigens, but the ability to retain these antigens undiminished in artificial culture; and secondly that in comparing vaccines for their immunizing potency passive protection tests in mice are more sensitive than active protection tests.

Alcoholized vaccine should be stored in the refrigerator. Under these conditions it appears to retain its power of stimulating Vi and O antibodies unimpaired for at least 10 years (Felix and Anderson 1951a). The keeping quality of vaccines preserved with 0.5 per cent. phenol or 0.3 per cent. tricresol is difficult to estimate. Judged by their ability to stimulate the production of agglutinins in rabbits or to protect mice against infection with living organisms, these vaccines, if stored at a temperature under 10° C., seem to undergo little deterioration in potency over a period of 3 years (McCoy and Bengtson 1920, Perry *et al* 1931a, Mishulow *et al* 1937, Babudieri 1939); though Felix and Anderson (1951b) found that a phenolized vaccine after storage for 10 years at 1-2° C. had lost the small power it originally possessed of stimulating the production of Vi antibodies.

In South Africa an endotoxoid T.A.B. vaccine has been extensively used, prepared according to the method described by Grasset and Gory (1927a, b) and Grasset (1931, 1935). This consists essentially in washing off a 48-hour agar growth with distilled water, killing the organisms by heat at 58° C. for 30 minutes, breaking them up by four successive heatings and freezings, and finally rendering the endotoxin so obtained non-toxic by incubation for a month in the presence of 0.7 per cent. formalin. The endotoxoid can be given in larger doses than vaccines of whole bacilli. According to Grasset (1938b) the vaccine was used for the inoculation of about 400,000 persons with more satisfactory results, judged by the diminution in the case incidence and case-fatality rates, than those previously obtained from ordinary T.A.B. vaccine. Grasset's claims seem to be borne out by the experience of the South African troops in the North African campaign, which fared as well as those inoculated with the British Army vaccine (Boyd 1943b).

Kourilsky, Kourilsky and Boivin (1939) tried to make a vaccine from the pure glycolipid somatic antigens of the typhoid bacillus, but were unable to reduce the toxicity of the preparation sufficiently. Morgan, Favorite, and Horneff (1943), however, reported success in the preparation of a suitable purified antigen.

Whatever vaccine is used it is desirable, if immunity is to be maintained at a high level, to re-inoculate subjects every 2 to 4 years. For this purpose one dose is sufficient.

Siler and Dunham (1939) of the American Army recommend a single dose of 0.1 ml. (100 million organisms) of the phenolized vaccine injected intradermally; according to their findings, this provides an adequate stimulus, and gives rise to less local and constitutional reaction than the larger dose which is usually given subcutaneously. The alcoholized vaccine is not suitable for intradermal injection.

advantages over parenteral injection. Vaccination by the oral route is seldom practiced.

Passive Immunization. The use of antityphoid serum for therapeutic purposes has been considered by a number of workers, but there is still no conclusive evidence as to its value. Whether or not "antiendotoxic" sera or other typhoid antisera confer a passive immunity to typhoid infection in man is likewise not established.

ALCALIGENES FECALIS

Alcaligenes fecalis or *Bacterium fecalis alcaligenes* closely resembles the typhoid bacillus morphologically, culturally and even in its growth on Endo, Conradi-Dragalski and malachite green differential media. It has been found in feces and in water. It differs from the typhoid bacillus in the possession of one or more polar, instead of many peritrichous, flagella, more luxuriant growth on potato with a brown coloration; and distinct alkali production in mannitol and litmus milk. It fails to produce acid from dextrose and other carbohydrates. It has been suggested that *Bact. alcaligenes* is a form of *Bact. fluorescens non-liquefaciens* which has lost the function of pigmentation, and it does have affinities with certain of the plant pathogens and soil bacteria. Its systematic position has been examined critically by Conn³¹ who has proposed a new genus, *Agrobacterium*, to include this bacillus together with *Bacterium radicola* and the bacterium of hairy root disease. Other species have been described as occurring in the intestinal tract, viz., *Alcaligenes metalcaligenes*, *Alcaligenes bookeri* and *Alcaligenes recti*, but these are not ordinarily differentiated from *Alcaligenes fecalis*. Other varieties, *Alcaligenes viscosus* and *Alcaligenes marshallii*, are found in dairy products and produce a slimy alkalinity and ropiness in milk.

Alcaligenes fecalis is only feebly pathogenic for experimental animals and presumably also for man. Human infection is observed in rare instances, however, in the form of mild typhoid-like disease, bacteremia and cystitis.

³¹ Conn Jour Bact, 1942, 44 353.

season to another. A considerable proportion of the eggs laid by such birds are infected with *Salm. pullorum*; Kaupp (quoted by Rice) records nearly 20 per cent. of infected yolks among 3,510 fresh eggs examined. Thus, in an infected flock, each year's hatchings produce a high proportion of infected chicks, and the cycle is repeated from year to year.

According to Severens, Roberts and Card (1944) genetic factors play an important part in determining susceptibility to the disease. During the first 5-10 days of life there is an increase in resistance to experimental infection, which is far greater in some chicks than in others. The increase is accompanied by a rise in the absolute and relative numbers of circulating lymphocytes. Evidence is brought to show that procedures such as splenectomy or irradiation with X-rays, which lower the lymphocyte count, lead also to a diminution in the resistance of the host.

The problem of control clearly resolves itself into the elimination of the carrier hens and this has been rendered possible by the application of the agglutination test. Blood is obtained from the wing vein, and the serum tested against suspensions of *Salm. pullorum*. This organism is non-flagellated, and contains only O antigens: the conditions must therefore be adjusted to reveal agglutination of this type. It is customary to use living suspensions, but it seems probable that alcoholized suspensions would prove as sensitive and could more readily be standardized. Incubation should be prolonged for 24 hours, though the results are usually readable after 4 hours at 50° C. The titre usually taken as diagnostic of infection is 1/40 to 1/50. Adopting this standard, it would appear that some 90 per cent. of carrier hens can be detected (Rice 1926). Such tests may be carried out at any time, and the detected carriers should be killed. The essential point, in preventing the perpetuation of infection from one season to another, is the elimination of all carriers among the birds preserved for breeding. The whole flock should be tested when the hatching season is over; those which show agglutinins should be killed, and those which give negative reactions should be retested. Only birds which have twice reacted negatively, and whose previous history gives no reason to suspect infection, should be retained for breeding in the following year.

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THE ENTERIC BACILLI: THE DYSENTERY BACILLI¹

Dysentery is a clinical rather than an etiological entity, and its characteristic symptoms, diarrhea, abdominal pain and blood in the stools, may occur either alone or as part of the syndrome of a number of diseases. In the former instance dysentery may be of protozoan (amebic dysentery, p. 755) or bacterial etiology. In addition there is evidence suggesting that a filterable agent, presumably a virus, can cause dysentery in man.² A dysentery-like infection may be produced by some members of the *Salmonella* group, but usually another group of bacteria, the dysentery bacilli, is responsible.

The dysentery bacilli are gram-negative, non-spore-forming rods related to the other enteric bacteria. Some of them resemble *Bact. coli anaerogenes* and the typhoid bacillus in that they ferment carbohydrates with the production of acid and no gas. Others are, perhaps, related to the slow or late lactose-fermenters designated as paracolon bacilli. None of the dysentery bacilli are motile, and hence they do not contain the two types of antigens found in the paratyphoid bacilli. As a group they differ from one another biochemically and immunologically. In general, their uncertain relationship to the other enteric bacilli, coupled with their own heterogeneity, has made their classification a difficult matter.

The dysentery bacilli are facultative anaerobes and their optimum temperature for growth is 37° C. Their nutritive requirements are not complex in that they will grow upon the ordinary nutrient (beef extract) media. In synthetic solutions nicotinic acid is apparently required by some strains, but whether amino acids are necessary is not known. They ferment glucose to much the same end products as the other enteric forms—lactic acid together with smaller amounts of formic and acetic acids and ethyl alcohol. Like the other gram-negative bacilli, they are relatively resistant to the bacteriostatic action of dyes, and these substances may be incorporated in differential media for their isolation; eosin-methylene blue agar is commonly used.

Classification. The dysentery bacilli are divided into two groups on the basis of the fermentation of mannitol, the non-mannitol fermenters including the Shiga bacillus. This distinction was of early importance and continues to be in tropical regions and elsewhere where the Shiga bacillus occurs, for the dysentery produced by it is much more severe and has a higher case fatality rate than the other bacillary dysenteries, but is not of practical signifi-

¹ See the general reviews by Neter. *Bact. Rev.*, 1942, 6 1, *ibid.*, *Gastroenterology*, 1943, 1:366, Weil. *Jour. Immunol.*, 1943, 46:13, *ibid.*, 1947, 55 363.

² Gordon, Ingraham and Korns. *Jour. Exp. Med.*, 1947, 86:409.

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cance in this country where there is little or no Shiga dysentery. In addition to the Shiga bacillus, this group also includes the so-called paratyphoid and the Schmitz bacillus. The group of non-toxic fermenters is further subdivided on the basis of slow (four to seven days) fermenters, of *longae*, and the fermentation of dulcitol and sorbitol. In all six groups, which are given species rank are determined on a biochemical basis.

In the past the nomenclature of the dysentery bacilli has been somewhat casual and informal, names such as the Shiga bacillus, the Flexner bacillus, the Strong bacillus, the Hay-Farrall bacillus, etc., have had wide currency. The name *Shigella* for the genus has gained considerably more acceptance and the dysentery bacilli are regarded by most workers as species of this single genus. The Bergey (1944) classification agrees with all the previous names, except in the case of the Sonne bacillus that have long been associated with these bacteria, this makes for some confusion, especially in the United States. It is this classification that must yield some degree of general acceptance. For present purposes the following will be regarded as species of the dysentery bacilli:

- (1) Non-toxic fermenters
 - Shigella flexner*
 - Shigella paratyphosa* (various serological types)
 - Shigella sonnei* (Schaller)
- (2) Toxic fermenters
 - (a) *Shigella flexner*
 - Shigella flexner* (but excluding several types)
 - Shigella disenteriae*
 - (b) *Shigella sonnei*
 - Shigella sonnei*
 - Shigella dysenteriae*

Some of these are immunologically heterogeneous and some heterogeneity and some have been divided into immunological types as indicated on biochemical variants.

DIFFERENTIAL IDENTIFICATION OF SPECIES OF THE DYSENTERY BACILLI

Species	Toxin	Lactose	Sucrose	Glucose	Sorbitol	Dulcitol	Indole
<i>Shigella flexner</i>	—	—	—	—	—	—	—
<i>Shigella paratyphosa</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—
<i>Shigella flexner</i>	—	—	—	—	—	—	—
<i>Shigella disenteriae</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—
<i>Shigella sonnei</i>	—	—	—	—	—	—	—

— most strains ferment, + most strains do not ferment

Shigella Shiga (The Shiga Dysentery). This was the first *Shigella* bacillus to be described.

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The Japanese bacteriologist, Shiga, found the bacterium which bears his name in a study of an epidemic of dysentery in Japan in 1898. The same microorganism was found by Kruse in Germany two years later, and *Shigella shigae* is known as Shiga's bacillus or the Shiga-Kruse bacillus. The Shiga bacilli are immunologically homogeneous, but anti-Shiga sera show some cross-reaction with some strains of *Shigella flexneri* and *Shigella ambigua*.

Sh. shigae differs from the other dysentery bacilli in its marked toxicity for man and experimental animals. Apparently two types of toxin are formed; an endotoxin closely bound to the cell substance and which is a polysaccharide-lipid-polypeptide complex³ that appears to have considerable immunizing activity as assayed by mouse protection, and an exotoxin found in filtrates of broth cultures, protein in nature and thermolabile. The exotoxin (neurotoxin) has an effect upon the nervous system and produces paralysis, while the endotoxin appears to act chiefly upon the alimentary tract. Although generally regarded as a true soluble toxin, the Shiga toxin is not as potent as the toxins of the diphtheria and tetanus bacilli; it has been prepared in purified form having an LD₅₀ dose for mice of 1 to 10 µg.⁴ Antitoxin to this soluble toxin may be produced, though not to the high titers obtained against other soluble toxins, which has marked protective effect in animal experiments. The therapeutic use of these sera has given encouraging results in some instances, but their value is not firmly established. The soluble toxin, but not the endotoxin, may be inactivated by formaldehyde and the toxoid used as an immunizing agent. It has not yet been possible to destroy the toxicity of this or other dysentery bacillus endotoxins and at the same time retain antigenicity.

Shiga bacillus infections have been observed most frequently in India, Japan, China and other parts of Asia; they appear to be relatively rare in the United States.

Shigella Parashigae. Strains of dysentery bacilli culturally identical with *Sh. shigae* but immunologically unrelated were found by Dudgeon and Urquhart⁵ in Macedonia in 1919 and designated by them *Bacterium parashigae* (—) in contrast to the Schmitz bacillus (see below) which they termed *Bacterium parashigae* (+). These bacilli have been observed from time to time in various parts of the world, including the United States, associated with diarrheal disease. They were studied in some detail by Large⁶ and by Sachs⁷ and are sometimes known as the Large-Sachs group or Sachs group of dysentery bacilli. Sachs distinguished eight immunological types but Wheeler and Stuart⁸ found that three of these were paracolon bacilli, and described an additional new type, making six valid types in all. The Sachs types are Q454, Q771, Q902, Q1030 and Q1167 and the Wheeler and Stuart type 1831.

Other non-mannitol fermenting dysentery bacilli which differ immuno-

³ Morgan and Partridge: *Biochem. Jour.*, 1940, 34:169.

⁴ Dubos and Geiger. *Jour. Exp. Med.*, 1946, 84:143.

⁵ Dudgeon and Urquhart: *Med. Res. Council Spec. Rept. Series No. 40*, 1919.

⁶ Large. *Jour. Roy. Army Med. Corps*, 1934, 63:80, 231.

⁷ Sachs. *Jour. Roy. Army Med. Corps*, 1943, 80:92.

⁸ Wheeler and Stuart. *Jour. Bact.*, 1946, 51:317.

CHAPTER 70

BACILLARY DYSENTERY

INTRODUCTORY

TOGETHER with plague, cholera, and influenza, dysentery has been one of the great scourges of the world. Though rapidly growing less common, it was at one time a fatal epidemic disease, dogging the footsteps of armies in the field, present in camp, gaol, and hospital, and decimating the white races in the tropics. "Wherever the general hygienic conditions are bad, wherever the soil is much fouled by excreta . . . , wherever many people are crowded together in one building or camp, where the food is coarse, monotonous or unsound, there, especially in tropical and sub-tropical climates," dysentery is liable to break out (Manson 1914).

Before the last quarter of the nineteenth century dysentery was regarded as a single, well-defined disease. In 1875, however, Loesch demonstrated the presence of parasitic amœbæ in the stools of dysenteric patients, and in 1883 Robert Koch observed these amœbæ in the intestinal wall of patients dying of dysentery in Egypt. The subsequent demonstration of amœbæ in liver abscesses of patients who were then suffering, or who had at some previous time suffered, from dysentery left little doubt that at any rate one of the causal agents of this disease was the *Entamœba histolytica*. In 1898-1901 Shiga in Japan, Flexner in the Philippines, and Kruse in Germany brought evidence to show that another type of the disease was due to a bacillus which was closely allied to *Salmonella typhi*. Dysentery may also at times be caused by protozoa other than *Entamœba histolytica*, such as *Balantidium coli* and *Bilharzia mansoni* (see Manson-Bahr 1942).

In this chapter we shall deal exclusively with bacillary dysentery, referring our readers to textbooks on tropical medicine and protozoology for descriptions of the amœbic and balantidial types.

Historical.—Dysentery affords an illuminating example of a case in which the agglutination reaction was successfully employed to discover the causative agent of a disease.

Studying the epidemic of dysentery in Japan in 1896, Shiga (1898) isolated from the faeces and intestinal wall of dysenteric patients a Gram-negative, non-gelatin-liquefying coliform bacillus. This organism, which was cultivated from 34 patients, differed from the common *Bact. coli* in its failure to coagulate milk, and in certain other cultural properties. At the same time nine other organisms were cultivated. To ascertain which of them, if any, was causally related to the disease, Shiga tested the action of the patient's serum on each one of them. Only one of the types was agglutinated. This type was, moreover, never found in the faeces of patients suffering from other intestinal diseases such as typhoid or simple diarrhoea, nor was it agglutinated by the serum of such patients, or by the serum

logically from *Sh. shigae* have been described. Of these the organism designated *Sh. arabinotarda* type A and Gøber and Stacy strain 8524 has been found to be identical with Q771, and *Sh. arabinotarda* type B with Q1167. Still another, *Sh. ualefeldi*, is a putacolon bacillus. At the present time, then, *Sh. paradysenteriae* appears to be immunologically heterogeneous and made up of six types which may be designated Type Q351, Type Q771, etc.

Shigella Ambigua (*Shmitz* bacillus, *Bacterium schmitzi*, *Bacterium ambiguum*). This species was described by Schmitz in 1917 as a cause of dysentery in a Rumanian war prison camp. Like *Sh. shigae*, it does not ferment mannitol but differs in that it produces indol and ferments sorbitol and rhamnose. The species is immunologically homogeneous except that, according to Boyd,⁹ freshly isolated strains contain two antigens, one of which is



Fig. 79. *Shigella flexneri*. Smear from a pure culture. Fuchsin, $\times 1050$.

lost on continued cultivation, and antisera prepared from stock strains may not agglutinate fresh strains too well. There is some cross reaction, perhaps to one quarter titer, with the Shiga bacillus but agglutinins are not reciprocally absorbed. *Sh. ambigua* has been found in Europe, India, the Sudan and elsewhere, and in the United States. It is not as common in this country as some of the other dysentery bacilli but is encountered with some frequency and was implicated in an extensive institutional outbreak of dysentery in New York¹⁰ and as an important cause of dysentery among chimpanzees.¹¹

Shigella Flexneri (*Bacterium paradyenteriae*, Pseudodysentery bacillus, *Shigella paradyenteriae*, Flexner's Bacillus, Hiss and Russell's "Y" Bacillus, Strong's bacillus). Soon after Shiga's discovery, Flexner, working in the Philippines, discovered other dysentery bacilli which for a time were not clearly differentiated Flexner's bacilli and those described by Strong and Musgrave in 1900 differed from *Sh. shigae*, however, both serologically and in the fermentation of mannitol. Attempts to subdivide the bacilli of the Flexner group by biochemical methods have not been successful; a wide

⁹ Boyd. Jour. Roy. Army Med. Corps, 1935, 64:289.

¹⁰ Schliefsstein and Coleman Jour. Inf. Dis., 1937, 61:257.

¹¹ Galton, Mitchell, Clark and Riesen Jour. Inf. Dis., 1948, 83:147.

natural conditions, 1 from a hospital kitchen was carrying *Sh. dysenteriae*, and 8 were carrying Flexner's bacillus. Manson-Bahr (1919) likewise isolated *Sh. dysenteriae* from the lower intestine of flies in Palestine. Though dysentery bacilli may be found occasionally in the intestinal contents of these insects, they do not appear to survive for more than a few days. The probability is that flies act mainly by carrying infective material on their feet rather than in their intestine.

It is doubtful whether under the modern sanitary conditions of western civilization flies play more than a small part in the transmission of either the epidemic or the endemic disease. In this country, as has been pointed out, dysentery is in fact more a winter than a summer disease. In the East and Middle East, however, as was shown again in the experience of the second world war, fly-borne infection is of major importance (Fairley and Boyd 1943); and even in the United States it is not unknown under military conditions (Kuhns and Anderson 1944).

Epidemic infection may result from the contamination of milk or of ice-cream; several well-authenticated outbreaks due to these vehicles have been recorded in different countries. Food, especially of the ready-cooked variety, is very susceptible to contamination from infected fingers and dust, and is of importance in the endemic spread of the disease. Occasionally dysentery is water-borne. For example, there are records of a large outbreak in Japan due to an aberrant dysentery bacillus affecting 11,000 persons and causing 500 deaths (Report 1938); of another in Kansas State due to *Sh. flexneri* affecting 3,000 persons (Kinnaman and Beelman 1944); of another in Wales due to *Sh. flexneri* W affecting 1,100 persons (Wade 1922), and of two smaller ones in England in which *Sh. sonnei* was isolated from the water itself (Green and Macleod 1943, Ross and Gillespie 1952). Water, however, seems to play little part in the high endemic prevalence in western countries. On the other hand, in Java, where peculiar conditions prevail, bacillary dysentery is said to be predominantly a water-borne disease (Fairley and Boyd 1943).

Infection is derived as a rule from cases, particularly ambulant cases, of the disease, from healthy convalescents, from symptomless carriers—usually temporary—and probably far less often, except in mental institutions, from chronic carriers. The organisms, which are excreted in the faeces, may gain access to food through the imperfectly cleansed fingers of the patient or carrier; or they may pass from one person to another by contact with inanimate articles such as seats, door handles, and water-flushing devices in lavatories, bed-pans and washing bowls in hospital wards, pencils and books in schools, and crockery and glasses in restaurants. There are in fact so many ways in which infection may spread that, once it is introduced into nurseries or children's wards in hospitals, it is often extremely difficult to eradicate. The use of the phage-typing method may help to clarify the mode of transmission of infection, particularly of Sonne's bacillus.

Bacillary dysentery varies greatly in its clinical manifestations and in its severity. In every outbreak there are a number of cases of simple diarrhoea, which are nevertheless caused by the dysentery bacillus. Entirely symptomless carriers are not uncommon, and in endemic areas may constitute a considerable proportion of those found to be infected (Watt and Hardy 1945). Even though the disease is often fatal in infants and very young children, it may be extremely mild and give rise to no more than a trivial disturbance of the bowel disappearing within 24 hours (Report 1943). Occasionally food-borne outbreaks, particularly of the Sonne type, are characterized by acute gastro-enteritis rather than by true dysentery (Rohleder 1938, Report 1942a). A very small number of cases of dysentery that recover,

variety may be separated on the basis of the variable fermentation of sucrose, dulcitol, sorbitol, maltose, raffinose, arabinose, inositol and salicin, and indol formation, but such varieties are not correlated with immunological type and have had little practical value.

Sh. flexneri is made up of a group of immunological types that are distinct and yet related to one another. Five immunological types were distinguished by Andrewes and Inman¹² on the basis of the distribution of four antigens, V, W, X and Z, which are designated as types V, W, X, Y and Z and whose antigenic composition is illustrated in Fig. 80. A number of other systems of typing *Sh. flexneri* were suggested but none gained the general acceptance of the Andrewes and Inman types.

Subsequently Boyd¹³ reported evidence of the presence of type- and group-specific antigens in these types and in additional related forms and suggested that numbered types be substituted for the Andrewes and Inman types. Further study by Wheeler¹⁴ indicated the pattern of distribution of antigens among the types suggested by Boyd with the modification that Type II be split into two subtypes, this is illustrated in the accompanying table. The

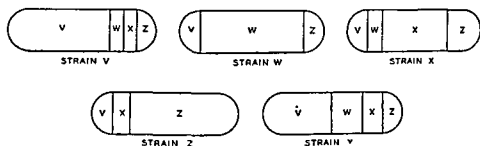


Fig. 80. Diagrammatic representation of the antigenic structure of the Andrewes and Inman types of *Sh. flexneri*.

basis of Boyd's contention that the O and Y types of Andrewes and Inman are not valid since they contain no type-specific antigen and are possibly to be regarded as degraded variants of Types IIa and IIb is clear from this. Of the Andrewes and Inman types accepted by these workers, V, W, and Z become Types I, II and III respectively. Types IV and V are new types, first found by Boyd in India and called by him 103 and P119 respectively.

Type VI, or Boyd 88, is not new but a variety of the Newcastle-Manchester bacillus which was first isolated in 1929 from cases of dysentery.¹⁵ The original Newcastle strain appears to be somewhat apart from the other dysentery bacilli in that it is feebly motile and produces gas, usually in small amount, in the fermentation of glucose. The original Newcastle strain did not ferment mannitol, but the variety known as the Manchester type does ferment this alcohol, some strains produce a small amount of gas and others do not. Almost all of the Newcastle-Manchester bacilli isolated in the United States have been of the mannitol-fermenting, non-gas-forming type. The variety known as Boyd 88 ferments mannitol but does not form gas, and about one-third of the strains

¹² Andrewes and Inman. Med. Res. Council Spec. Rept. Series No. 42, 1919.

¹³ Boyd. Jour. Hyg., 1938, 38:477, Trans. Roy. Soc. Trop. Med. Hyg., 1940, 33:553.

¹⁴ Wheeler. Jour. Immunol., 1944, 48:87.

¹⁵ Clayton and Warren. Jour. Hyg., 1929, 28:355, *ibid.*, 1929, 29:191.

That is to say, Flexner's bacillus was half as common again as *Sh. dysenteriae*. But Graham (1918) and his co-workers, who investigated 2,500 cases of dysentery and diarrhoea in the British Salonika Force at much the same time—June, 1916, to the end of 1917—found that of the severe and moderately severe bacillary dysenteries 75 per cent. were due to *Sh. dysenteriae* and only 25 per cent. to *Sh. flexneri* Y, whereas of the mild bacillary dysenteries 75 per cent. were due to *Sh. flexneri* Y and only 25 per cent. to *Sh. dysenteriae*.

The figures given by Boyd (1936) in India for 1932-4 showed that just over half the strains isolated were of the classical Flexner type, and that about 13 per cent. belonged to newly recognized Flexner or Boyd types; Shiga's bacillus constituted about 15 per cent., Schmitz's bacillus 5 per cent. and Sonne's bacillus

TABLE 129

SPECIES AND TYPES OF DYSENTERY BACILLI ISOLATED IN INDIA 1932-4, AND IN THE MIDDLE EAST FORCE BETWEEN AUGUST 1940 AND JUNE 1943.
(Modified from Boyd 1936, 1946)

Type of Bacillus.	India.		Middle East Force	
	No of Straths	Per cent.	No of Straths	Per cent.
<i>Sh. dysenteriae</i>	820	14.9	4,516	18.9
<i>Sh. schmitzi</i>	296	5.4	1,601	6.7
<i>Sh. flexneri</i> 1-3 with X and Y variants . . .	2,888	52.6	14,752	61.6
" " 4a	135	2.5		
" " 5.	100	1.8		
" " 6.	240	4.4		
<i>Sh. boydi</i> 1	134	2.4	516	2.2
" " 2	26	0.5		
" " 3	41	0.7		
" " 4	47	0.9		
" " 6	5	0.1	800	3.3
Other non-mannitol fermenters	—	—		
Other mannitol fermenters	152	2.8	1,766	7.4
<i>Sh. sonnei</i>	508	9.3	—	—
Inagglutinable lactose and sucrose fermenters . . .	97	1.8	—	—
<i>Entamoeba histolytica</i>	965	—	3,463	—

9 per cent. of the total strains studied. Similar proportions were observed in the Middle East Forces during the second world war (Table 129).

In the United States Shiga's bacillus seems to be uncommon. Hardy, Watt, Kolodny and DeCapito (1940), who isolated 2081 strains of dysentery bacilli from cases of diarrhoea and dysentery in New Mexico during the years 1936, 1937, and 1938, did not meet with it once. They identified 65.0 per cent. of their strains as *Sh. flexneri*, 19.1 per cent. as *Sh. sonnei*, and 15.9 per cent. as *Sh. newcastle*. Shiga's bacillus is, in fact, seldom met with except in tropical countries and East Asia (Weil 1943).

Clinically the disease due to Shiga's bacillus is much more severe than that due to Flexner's; as well as the abdominal pain, diarrhoea and tenesmus, there is often a profound toxæmia ending in collapse. Complications such as arthritis (Graham 1919, Report 1919) and iridocyclitis, when they do occur, are almost invariably associated with the Shiga type of infection. Moreover, recovery from

do not ferment dulcitol, and the remainder are late dulcitol fermenters. The Newcastle Manchester bacilli are all late dulcitol fermenters and produce a small amount of gas. The group is, however, immunologically homogeneous and related to the Flexner bacilli and may not unreasonably be regarded as a type of *Sh. flexneri*.

ANTIGENIC STRUCTURE OF *SH. FLEXNERI* TYPES*

Andrews and Inman and Boyd Types	Antigenic Structure		Type
	Type Specific	Group Specific	
V	I	1, 2, 4, 5, 6, 9	I
W	II	1, 3, 4	IIa
	II	1, 7, 8, 9	IIb
X	none	1, 7, 8, 9	
Y	none	1, 3, 4	
Z	III	1, 6, 7, 8, 9	III
103	IV	1, 6	IV
P119	V	1, 5, 7, 9	V
88	VI	1, 2, 4	VI

* According to Whittet Jour. Immunol., 1944, 46 87

Other mannitol fermenting dysentery bacilli have also been described by Boyd as 170, P288, D1, D19, P143 and P274. These are not related immunologically to the Flexner bacilli, and it has been suggested that they be named *Sh. boyd* Types I, II, III, IV, V and VI respectively. They are, however, included by Weil, Black and Farsetta¹⁶ with the Flexner dysentery bacilli. These workers accept Types I to VI of *Sh. flexneri* as defined by Boyd but add the Andrews and Inman types X and Y as Types VII and VIII. The Boyd types a

XIV inclusive; Type IX is

170 or

Still giving other biochemical reactions typical of *Sh. flexneri*, but immunologically unrelated, have been included in this species. A variety found in the Mediterranean area and named *Sh. etousae* has been included as *Sh. flexneri* Lavington I, and Francis,¹⁷ disregarding Weil's numbering of types, has suggested that two varieties immunologically related to the Andrews and Inman types be provisionally typed as Types VII and VIII.

It seems clear that this somewhat confused state stems from the lack of a reasonably precise, generally accepted, working definition of *Sh. flexneri*. If it be made on a purely biochemical basis, the extended series of types of Weil, Black and Farsetta should logically stand, to be amplified as other immunological varieties of the biochemical type are described. If, however, the definition of the species is also to call for an immunological interrelationship, only

¹⁶ Weil, Black and Farsetta: Jour. Immunol., 1944, 49 321.

¹⁷ Francis: Jour. Path. Bact., 1946, 58 320.

Boyd's Bacillus.—The various types of bacilli described by Boyd, though less frequent as a cause of dysentery than Flexner's or Sonne's bacilli, must be regarded as pathogenic for man (Boyd 1939-40, Rothstadt *et al.* 1942). *Sh. etousae*, which is now known as *Sh. boydi* Type 7, was responsible for a large outbreak of dysentery in a mental hospital in England (Lavington *et al.* 1946), and for an outbreak among United States troops in Casablanca during the second world war (Stock *et al.* 1947).

Sonne's Bacillus.—This is a more important organism. Though it had undoubtedly been previously observed in the United States and Germany, it was Sonne's description in 1915 that drew serious attention to it. From a number of sporadic cases of dysentery in Denmark Sonne cultivated a bacillus differing from *Sh. flexneri* in its late fermentation of lactose, in its striking production of acid in litmus milk during the second week of incubation, and in its different serological reactions. In an investigation of dysentery in Norway, Thjøtta (1919) found that 25 out of 65 strains isolated conformed to the Sonne type; and in Denmark Bojlén (1934) found it to be as common as Flexner's bacillus in the causation of endemic and institutional dysentery. In this country the organism has been recognized since about 1925 (see Channon 1926, Kerrin and Cruickshank 1926), though there is reason to believe that it was present but not identified before this date. During the second world war it was very prevalent, and in the five years following the war it was responsible for about 80 per cent. of the diagnosed cases of dysentery. It is widespread in North America (see Johnston and Brown 1930, Nelson 1930, Leahy 1931, Felsen and Osofsky 1934, Weil 1943, Watt and Walton 1949) and has been observed in several other parts of the world. It is most frequently met with in asylums, children's hospitals, residential and day nurseries, and nurses' homes. Adults, however, are not spared, and in household outbreaks may often constitute a quarter of the infected cases. They tend to be symptomless excretors more often than children. In infants Sonne's bacillus may give rise to an acute toxic form of dysentery, which is known in Japan as *Eki-ri*; and it may be associated with fatal cases of convulsions (Lewis and Claireaux 1951). The disease often occurs in epidemic form, and several milk-borne outbreaks have been described (see Sylvest 1933, Bojlén 1934, Felsen and Osofsky 1934, Bowes 1938, Faulds 1942, 1943). Leuchs and Heim (1930) reported an outbreak due to infected cheese affecting 51 persons. Clinically, Sonne dysentery is said by some observers to be less severe than Flexner dysentery, but Bojlén (1934) disputes this. Mild and atypical cases of the disease are common in any reasonably large outbreak. As has already been mentioned, Sonne's bacillus often gives rise to acute gastro-enteritis rather than to dysentery proper. Rohleder (1938), who analysed 118 cases in which this bacillus was demonstrated, found that 72 of them had run a clinical course indistinguishable from that of a simple gastro-enteritis. Bacteriologically, the organisms are found in the faeces, and occasionally in the urine (Rohleder 1938), agglutinins are often demonstrable in the blood serum of patients suffering or convalescent from the acute disease. The closely related organism, *Bact. dispar*, has so far not been definitely incriminated as a cause of dysentery, but further observations are required (see Forsyth 1933). The fact that some indole-positive strains were isolated by Felsen and Osofsky (1934) and Beck and Buckle (1939) from hospital outbreaks of dysentery suggests that this organism cannot be entirely dismissed as a possible cause of the disease.

Other Organisms.—A few other organisms may give rise to a disease diagnosed as dysentery. For example, *Bact. alkalescens*, which used to be classified with

Types I to VI are valid. The present tendency, which is not completely consistent, is to continue to accept the Andrewes and Inman types and regard the Newcastle-Manchester bacilli as somewhat apart though immunologically related, or to accept the first six types only. As for the remainder, only the British as yet recognize *Sh. boyd* Types I to VI, and in this country these are designated *Shigella* sp. 170, *Shigella* sp. P288 and so on.

All of the Flexner bacilli are of world-wide distribution, the relative proportions varying from one locality to another. As a whole *Sh. flexneri* is found in a large proportion of the dysentery cases in temperate climates, and even in tropical countries they are perhaps the most common of the dysentery bacilli. In this country, for example, 451 strains of a total of 769 isolated in routine examinations in Connecticut in 1940-43 were *Sh. flexneri*, and of 1329 dysentery bacilli isolated from British and Indian troops in India, 999 were Flexner, 197 Shiga, 100 Schmitz and 33 Sonne bacilli.

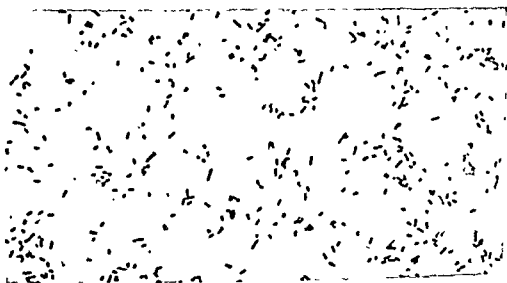


Fig. 81. *Shigella alkalescens*. Smear from a pure culture. Fuchsin, $\times 1050$.

Sh. flexneri forms no soluble or exotoxin but does contain an endotoxin. It has been studied in some detail by Goebel and his co-workers¹⁸ who have found that the somatic antigen which contains the toxicity is made up of a lipid component, a protein component, two carbohydrate components one of which contains labile acetyl groups, and a toxic component which Goebel believes to be a distinct substance and possibly associated with a purine or pyrimidine-like substance. In general, it has not been possible to detoxify the endotoxin without destroying its antigenicity, though Barnes *et al.*¹⁹ have reported that this can be accomplished by treatment with heat or ultraviolet light in the presence of an oxidizing agent and caprylate ion.

Shigella Alkalescens. *Shigella alkalescens* was described by Andrewes in 1918. Unlike the other dysentery bacilli, these bacilli ferment dulcitol. For some time they have been regarded as of uncertain pathogenicity but evidence is accumulating which indicates that they are associated with disease. They

¹⁸ Goebel *et al.*: Jour. Bact., 1944, 47:476; Jour. Exp. Med., 1945, 81:315, 331, *ibid.*, 1947, 85:499.

¹⁹ Barnes *et al.*: Jour. Immunol., 1947, 56:255.

Bojlén (1934) was struck by the frequency of persistent carriers. Some patients, whose stools were systematically examined, were found to excrete the bacilli for years. The experience of most workers, however, shows that chronic carriers—that is, those who excrete the organism for a year or more—are infrequent, and therefore play but a small part in the spread of the disease, except perhaps in mental institutions.

It is sometimes stated that there are no healthy carriers of dysentery bacilli, and that all those who excrete the organism are suffering from chronic dysenteric lesions (see Scott 1938). The evidence in favour of this view is far from convincing. Several workers have demonstrated the presence of *Sh. flexneri*, *Sh. sonnei*, *Sh. schmitzi*, and even *Sh. dysenteriae* in completely symptomless carriers (see Report 1942b, d, Weil 1943). It is possible that before the introduction of deoxycholate citrate medium, cultures were successful only from heavily infected persons; but now that even small numbers of dysentery bacilli may be demonstrated, the existence of perfectly healthy carriers is no longer in doubt. Hardy, Watt and DeCapito (1942), for example, found that in an endemic area as many as 3.8 per cent. of persons who gave no history of illness during the preceding year were "passive carriers" of the dysentery bacillus. Doubtless they had been in contact with other carriers or cases, and had become infected without developing the disease.

The introduction of S.S. and deoxycholate media has also led us to modify somewhat our ideas on intermittency of excretion. Even so, it is not uncommon to observe intermittency in convalescents and carriers, though less often than formerly (see Cruickshank and Swyer 1940, Report 1942e). The apparent duration of excretion has been extended by the use of these media. Most workers would have been prepared to accept Fletcher and McKinnon's (1919) findings that not more than 2 to 3 per cent. of convalescents carried the bacillus for over three months. Watt, Hardy and DeCapito (1942), however, working with S.S. and with deoxycholate citrate media, found that 11 per cent. of Flexner, 10 per cent. of Newcastle, and 7 per cent. of Sonne carriers excreted the bacillus for over ten weeks.

In conclusion it is worth while comparing dysentery with other enteric infections, notably typhoid. Dysentery has a short incubation period, about 48 hours as a rule. It is essentially a local disease; the bacilli remain confined to the intestine and to the mesenteric glands; they do not invade the blood stream, nor are they, except occasionally, found in the spleen or liver. In this respect dysentery—of the Shiga type—has been compared to diphtheria; the bacilli produce a necrosis of the intestinal mucosa, often with pseudo-membrane formation, and their toxic products are absorbed, giving rise to profound systemic disturbance. The disease may also run a chronic course. Typhoid fever, on the other hand, has a long incubation period—up to 21 days; it is primarily a septicæmic disease with secondary localization in the intestine, the bacilli do not produce a powerful toxin; and the disease has little tendency to become chronic.

Laboratory Infection in Man.—It has been pointed out in Chapter 29 that, with the possible exception of monkeys, dysentery cannot be reproduced in laboratory animals. There are, however, a few definite cases recorded in the literature of accidental infection in human beings. Kruse (1901) mentions the case of Dr. Stöcker, an assistant in his laboratory at Bonn, who apparently infected himself while working with cultures of Shiga's bacillus. He suffered from a mild attack

have been found associated with sporadic enteric disease, and a small epidemic has been reported recently.²⁰ In the last few years *Sh. alkalescens* has been recognized more and more frequently in this country, and appears to be more common than had been supposed. It is also pathogenic for experimental animals.²¹

Strains of *Sh. alkalescens* have been regarded as biochemically distinct and immunologically homogeneous. Stuart, Rustigan, Zimmermann and Corrigan,²² however, have found that the species is made up of a graded series of biochemical types. They have also demonstrated the presence of five antigens, two major antigens designated A and B, and three minor antigens, C, D and E, in these bacilli. A, B and C are present in all typical strains, while D and E occur singly or in combination to give four subtypes. They are immunologically related to the colon bacilli through the paracolon group and to Boyd P274. Two immunologically unrelated types have been described²³ and designated type II and type III in distinction to the original or type I. Weil and Sifkovsky²⁴ have suggested that type II be *Shigella tieté*. Both of these types include strains which ferment lactose. Whether these should be classified as *Sh. alkalescens* is possibly open to some question.

Shigella Sonnei (Sonne's Group III, Duval's bacillus). Lactose fermenting dysentery bacilli discovered by Duval in 1904 have since been rediscovered by quite a number of observers. By force of usage these bacteria have come to be known as the Sonne type. The Sonne bacillus ferments mannitol and does not produce indol; it is serologically distinctive and homogeneous. Glynn and Starkey²⁵ have reported, however, that there are two immunological types of *Sh. sonnei* which they designate I and II. Type I contains one antigen in predominance, while type II contains both antigens in equal amounts. The difference in agglutination titers is of practical importance and type II antiserum is the one of choice. The lactose fermentation is slow and may be delayed for a week or ten days, and strains of this type were doubtless confounded with Flexner bacilli by the earlier workers. It is probably considerably more common in the United States than appears in the records. It has also been reported in warmer countries, and perhaps a more detailed study of the differentiation of the mannite-fermenting bacilli would increase the amount of dysentery properly ascribable to this micro-organism.

immunological homogeneity tends to set them off.

Shigella Dispar. Other lactose-fermenting dysentery bacilli were designated *Bact. dispar* by Andrewes. Some of Andrewes' strains were *Sh. sonnei*, but the type now termed *dispar* differs from the Sonne type in that *saliva* is fermented and indol is formed. *Sh. dispar* is serologically heterogeneous.

²⁰ Felsen and Wolarsky: New York State Jour. Med., 1940, 40:1303, Stuart et al. Jour. Immunol., 1943, 47:425.

²¹ Edward: Jour. Path. Bact., 1940, 51:245.

²² Stuart, Rustigan, Zimmermann and Corrigan: Jour. Immunol., 1943, 47:425.

²³ Aris: O Hospital, 1933, 15:447, 5. Proc. Soc. Exp. Biol. Med., 1944, 57:200.

²⁴ Weil and Sifkovsky: Jour. Bact.

²⁵ Glynn and Starkey: Jour. Bact.

1942, Pot 1942, Report 1942f). The brilliant green method, which is of such value in the isolation of *Salmonella*, is of no help in the cultivation of dysentery bacilli, since these organisms are as susceptible as *Bact. coli* to the dye (Krumwiede and Pratt 1914, Myers and Koser 1932). Colonies should be picked off into lactose broth, put through the sugars, and tested by the tube or the slide method against specific agglutinating sera of the types most likely to be met with in the area concerned. Slide agglutination must be used with care, particularly for colonies off selective media, since non-specific results are common. Shiga's bacillus on first isolation may be poorly agglutinable; heating for 1 hour at 60° C. is said to improve it (Schütze 1914). *Sh. flexneri* 6 (Newcastle) does not ferment mannitol and may form no visible gas; non-mannitol-fermenting strains of dysentery bacilli, therefore, that are not agglutinated by an antiserum to Shiga or Schmitz, should be tested against a *flexneri* 6 antiserum (Ewing and Taylor 1915, Nelson 1917). *Proteus morgani* may similarly cause confusion, as it may be practically non-motile on isolation and form little or no gas; its identity can be determined by its ability to hydrolyse urea. Occasionally Flexner's bacillus may be in the group phase when first cultivated from the stools and its exact classification may present difficulty. In examining suspected dysentery bacilli, a motility test, preferably at 22° C., should never be omitted (Braun and Weil 1928). When isolated from fresh cases Sonne's bacillus is usually in Phase i, but in carriers Phase ii is generally found. For purposes of identification antisera against both phases must be available. Phage-typing may be carried out by the method of Hammarstrom (1947, 1949). For the laboratory diagnosis of amœbic dysentery, reference may be made to a paper by Hill (1947).

Agglutinins appear in the patient's serum about the 6th to the 12th day, but sometimes not till later, or not at all. The serum should be tested against standard suspensions of the common types of dysentery bacilli. The tubes are best incubated in a water-bath at 50° C. for 4 to 6 hours, left overnight at room temperature, and read finally the next morning.

Much less attention can be paid to the presence of serum agglutinins in the diagnosis of dysentery than in that of enteric fever. This is partly because normal human serum may contain agglutinins for dysentery bacilli, and partly because the group agglutinins may be so prominent as to render the diagnosis of the type of bacillus almost impossible. The value of the test depends to a considerable extent on the frequency distribution of normal agglutinins in the population. In endemic areas and in mental institutions in which dysentery is common, interpretation of low serum agglutinin titres is very difficult and often impossible (see Dudgeon 1919). On the other hand, in areas in which dysentery is uncommon, and especially in children, whose serum is usually devoid of normal agglutinins, the test may be of value. Under these conditions a titre of 1/25 or over to *Sh. dysenteriae*, *Sh. schmitzi*, or *Sh. sonnei*, and a titre of 1/100 or over to *Sh. flexneri* is highly suggestive of active infection. Except to *Sh. flexneri*, the agglutinin titre seldom rises high, and often declines in early convalescence. In Sonne infections, as a rule, only about 60 per cent., of patients develop serum agglutinins (Smith and Fraser 1930, Johnston and Brown 1930, Nabarro and Signy 1932, Laws 1936, Cruickshank and Swyer 1940); though a positive result therefore may be diagnostic, a negative result does not exclude the presence of active disease. In chronic cases agglutinins may persist for a long time.

Diagnosis at autopsy should be made by taking cultures from the mucosa and

and not related to *Sh. sonnei* but appears to be related to certain Flexner strains. Carpenter²⁶ found that a group of 37 strains could be separated into three immunological types, two related to one another and the third independent. *Sh. dispar* may be divided into two varieties, *Sh. dispar* var. *ceylonensis* which ferments dulcitol, and *Sh. dispar* var. *madampensis* which does not; Bergey (1948) gives these varieties species rank as *Sh. ceylonensis* and *Sh. madampensis*.

Pathogenicity for Man. The serum of patients suffering from acute dysentery agglutinates one or another type of dysentery bacillus in high dilutions. This fact and the constant occurrence of the same type of bacillus in the stools strongly suggest a causal relation in spite of the fact that the dysentery bacilli cannot, like the typhoid bacillus, be cultivated from the blood of the patients. Laboratory infection with a pure culture of the Flexner bacillus has occurred.²⁷

The incubation period of bacillary dysentery is generally short, about forty-eight hours, and the disease may be acute or tend to run a chronic course. Apart from the inflammatory, sometimes ulcerative or diphtheritic, lesions in the intestine (ulcerative colitis), the anatomical picture of dysentery presents little that is characteristic. The liver abscesses that are found, as a rule, in amebic dysentery are absent in the bacterial diseases, one series having been reported of 1130 cases of bacillary dysentery without a single abscess. Dysentery bacilli are sometimes found in immense numbers in the dejecta, often in almost pure culture. They may be found at autopsy in the mesenteric glands, but, as a rule, not in the spleen or other internal organs, nor do they commonly occur in the blood or urine. Bacillary dysentery is, therefore, not a septicemia but an infection localized in the alimentary tract, in this respect resembling Asiatic cholera rather than typhoid fever. Recurrent diarrheal disease, often called chronic ulcerative colitis, is frequently caused by dysentery bacilli, indicating that the bacilli may persist in the bowel, possibly in the superficial layers of the intestinal epithelium, for long periods of time.

The dysentery toxin is excreted in rabbits, and probably in man, by the large intestine. The selective action of the toxin upon the tissues, rather than any local action of the bacilli themselves, appears to be responsible for the inflammation and other local changes. When the toxin is introduced directly into the gut, no symptoms are produced, suggesting that the toxin primarily affects the deeper cells rather than the surface of the mucous membrane.

In the large series of cases studied in Denmark caused by the Sonne and Flexner types the case fatality was about 2 per cent. The Shiga bacillus dysentery of the tropics is much more often fatal (20 per cent). The clinical disease is considerably more severe in the Shiga infections than in Flexner infections, and complications, such as arthritis, are almost invariably associated with the former type of infection.

The relation of dysentery bacilli to summer diarrhea of infants is obscure. Some investigators have isolated the dysentery bacilli from the excreta, particularly in those cases where there is mucus in the stools. Those cases with which dysentery bacilli are associated do not appear to differ clinically from

²⁶ Carpenter: Proc. Soc. Exp. Biol. Med., 1943, 53:129.

²⁷ Lippincott: Jour. Amer. Med. Assn., 1925, 85:901.

1942, Pot 1942, Report 1942f). The brilliant green method, which is of such value in the isolation of *Salmonella*, is of no help in the cultivation of dysentery bacilli, since these organisms are as susceptible as *Bact. coli* to the dye (Krumwiede and Pratt 1914, Myers and Koser 1932). Colonies should be picked off into lactose broth, put through the sugars, and tested by the tube or the slide method against specific agglutinating sera of the types most likely to be met with in the area concerned. Slide agglutination must be used with care, particularly for colonies off selective media, since non-specific results are common. Shiga's bacillus on first isolation may be poorly agglutinable; heating for 1 hour at 60° C. is said to improve it (Schutze 1914). *Sh. flexneri* 6 (Newcastle) does not ferment mannitol and may form no visible gas; non-mannitol-fermenting strains of dysentery bacilli, therefore, that are not agglutinated by an antiserum to Shiga or Schmitz, should be tested against a *flexneri* 6 antiserum (Ewing and Taylor 1915, Nelson 1917). *Proteus morgani* may similarly cause confusion, as it may be practically non-motile on isolation and form little or no gas; its identity can be determined by its ability to hydrolyse urea. Occasionally Flexner's bacillus may be in the group phase when first cultivated from the stools and its exact classification may present difficulty. In examining suspected dysentery bacilli, a motility test, preferably at 22° C., should never be omitted (Braun and Weil 1928). When isolated from fresh cases Sonne's bacillus is usually in Phase i, but in carriers Phase ii is generally found. For purposes of identification antisera against both phases must be available. Phage-typing may be carried out by the method of Hammarström (1917, 1919). For the laboratory diagnosis of amœbic dysentery, reference may be made to a paper by Hill (1917).

Agglutinins appear in the patient's serum about the 6th to the 12th day, but sometimes not till later, or not at all. The serum should be tested against standard suspensions of the common types of dysentery bacilli. The tubes are best incubated in a water-bath at 50° C. for 4 to 6 hours, left overnight at room temperature, and read finally the next morning.

Much less attention can be paid to the presence of serum agglutinins in the diagnosis of dysentery than in that of enteric fever. This is partly because normal human serum may contain agglutinins for dysentery bacilli, and partly because the group agglutinins may be so prominent as to render the diagnosis of the type of bacillus almost impossible. The value of the test depends to a considerable extent on the frequency distribution of normal agglutinins in the population. In endemic areas and in mental institutions in which dysentery is common, interpretation of low serum agglutinin titres is very difficult and often impossible (see Dudgeon 1919). On the other hand, in areas in which dysentery is uncommon, and especially in children, whose serum is usually devoid of normal agglutinins, the test may be of value. Under these conditions a titre of 1/25 or over to *Sh. dysenteriae*, *Sh. schmitzi*, or *Sh. sonnei*, and a titre of 1/100 or over to *Sh. flexneri* is highly suggestive of active infection. Except to *Sh. flexneri*, the agglutinin titre seldom rises high, and often declines in early convalescence. In Sonne infections, as a rule, only about 60 per cent., of patients develop serum agglutinins (Smith and Fraser 1930, Johnston and Brown 1930, Nabarro and Signy 1932, Laws 1936, Cruickshank and Swyer 1940); though a positive result therefore may be diagnostic, a negative result does not exclude the presence of active disease. In chronic cases agglutinins may persist for a long time.

Diagnosis at autopsy should be made by taking cultures from the mucosa and

those in which they are not found, and it is uncertain just what proportion of cases of infant diarrhea is caused by the dysentery bacilli.

Carriers. It is probable that dysentery bacillus infection is very common, many of the infections going unrecognized because of the mildness of the symptoms. Persons with such infections are, of course, convalescent carriers who continue to discharge the bacilli for an average of three to five weeks. It is not clear whether a permanent chronic carrier state analogous to the chronic typhoid bacillus carrier occurs, but many convalescents continue to discharge dysentery bacilli over long periods of time and inapparent infection is not uncommon. In any case, it is the casual carrier and changing groups of casual carriers or ambulatory cases that are of primary importance in the maintenance and spread of the infection.

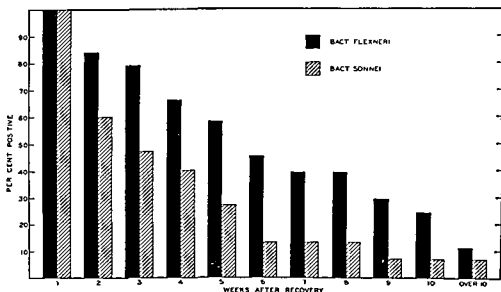


Fig 82 The persistence of dysentery bacilli in the feces of convalescents Prepared from data of Watt, Hardy and DeCapito Pub Health Repts., 1942, 57 524.

Bacteriological Diagnosis of Bacillary Dysentery. Since, as indicated earlier, dysentery is a clinical rather than an etiological entity, the causative microorganism must be isolated and identified to allow a diagnosis of bacillary dysentery. The bacilli may be found in fecal specimens and rectal swabs are cultured for the detection of carriers. The methods of enrichment culture and direct plating are essentially those used in the isolation of *Salmonella* and the typhoid bacillus. Desoxycholate-citrate and S-S agars are the most useful of the agar media; bismuth-sulfite is not suitable because species other than *Sh. flexneri* are inhibited. A tellurite-iron rosolic acid medium has been developed by Wilson and Blair²⁸ which is said to give good results,²⁹ but has not as yet been generally used. Colonies are picked and identified by fermentation reactions and slide agglutination; the latter may include typing of the Flexner strains.

²⁸ Wilson and Blair: Brit. Med. Jour., 1941, ii. 501.

²⁹ Thomas and Hulme: Lancet, 1942, ii 321.

soft X-rays (Moore and Kersten 1936), or periodic acid (Goebel 1947). Shiga (1903) advocated the use of a sero-vaccine, the first dose of which was followed in 4 days by an inoculation of bacilli alone. Antibodies were demonstrable in the blood of inoculated persons 3 to 4 weeks later. This method was used on about 10,000 persons in Japan during the years 1898-1900, and is said to have had little effect on the morbidity, but to have decreased the fatality considerably. Unfortunately Shiga gave no exact figures of the results. Olitsky (1918) suggested the use of a lipo-vaccine consisting of heat-killed bacilli suspended in neutral almond oil, on the principle that, owing to slower absorption of the toxin, the patient's reaction should be correspondingly less, but the vaccine gives rise to subcutaneous abscesses (Hardy, DeCapito and Halbert 1948). Iguchi, Ohstubo and Eguchi (1933) tried oral vaccination with apparently satisfactory results; these, however, still await confirmation. Boivin and Mesrobian (1938) recommended the use of a glycolipid antigen extracted from Shiga's bacillus, and Morgan and Schutze (1943) a phenol extract of the somatic antigen. No satisfactory or convincing evidence has yet been brought to show that in practice any of these vaccines is able to contribute significantly to the prophylaxis of Shiga dysentery. Formol toxoid is said by O'Brien (1939-40) to be a good antigen for horses, but it seems doubtful from the work of Steabben (1943) whether it could be relied on to protect against infection with living bacilli.

Hardy, DeCapito and Halbert (1948) carried out a careful study on the prevention of endemic bacillary dysentery in institutions in New York and Illinois. Neither a monovalent vaccine against *Sh. flexneri* Type 3 where this organism alone was responsible, nor a polyvalent Flexner, Sonne and Schmitz vaccine where multiple species and types were met with, had any appreciable effect on the attack rate or the severity of the disease as judged by comparison with unvaccinated control persons. Similarly, observations made on human volunteers who were experimentally infected with dysentery bacilli by the mouth showed that a polyvalent Flexner, Sonne and Schmitz vaccine, whether killed by ultraviolet irradiation or by heat, had no significant protective effect (Shaughnessy *et al* 1946). Whatever the reason, it must be concluded that vaccination can at present play no useful part in the prevention of dysentery.

Chemoprophylaxis.—Sulphonamide prophylaxis is sometimes used in the control of institutional outbreaks. Hardy (1946) recommends this method in Flexner and Schmitz infections if 10 per cent or more of the inmates are infected; he gives 1 gm. of sulphadiazine twice daily for 7 days. There is always a danger, however, of breeding sulphonamide-resistant strains, and it is probably wise, at any rate in mental institutions and residential nurseries, to use this method sparingly.

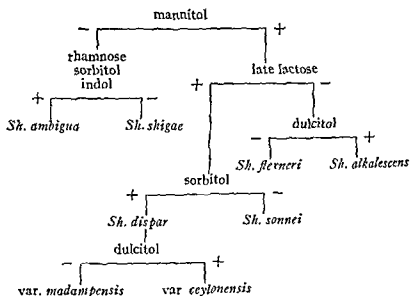
Serotherapy.—Todd (1904) working in London, and Rosenthal (1903, 1904) working in Moscow, showed independently that the soluble toxin contained in cultures of *Sh. dysenteriae* was able to give rise on injection into animals to a true neutralizing antitoxin. Todd found that the serum had definite protective properties; if it was injected intravenously into a rabbit, it protected the animal against a lethal dose of toxin or of dead bacilli, given simultaneously or half an hour later. A horse serum that he prepared contained sufficient antitoxin per ml. to neutralize 20,000 lethal doses of toxin for a rabbit. The union of toxin and antitoxin appears to take place in constant proportion (Pfeiffer and Ungermann 1909, Kolle *et al.* 1924).

Antiserum is prepared by the intravenous injection of horses, either with heat-killed and later with living bacilli (Flexner and Amoss 1915) or with formol toxoid.

The titration of the serum is best carried out on the mouse, using intravenous inocula-

Epidemiology. Infections caused by dysentery bacilli are probably far more common than is generally recognized. In 1945, 33,495 and 400 deaths were reported from 38 states, rates of 32.4 and 0.4 per 100,000 respectively. Attacks of severe illness grade off into mild and almost trivial attacks of simple diarrhea. Several outbreaks of typical "food poisoning" attributed to the Sonne bacillus are on record. In a number of localities where careful bacteriological studies have been made, dysentery bacilli have been found widely distributed both in patients with gastro-intestinal derangements and in the general population. Probably the most important single reservoir of infection is the human carrier, either convalescent or with inapparent infection. The extent to which the carrier state occurs has been appreciated only in recent years. In a study of dysentery bacillus infection in the normal population Watt and Hardy³⁰ found *Sh. flexneri* in 11 per cent of the population in New Mexico, 4 per cent

BIOCHEMICAL SEPARATION OF THE DYSENTERY BACILLI



in Puerto Rico, 3 per cent in Georgia and 0.1 per cent in New York City, with an estimated annual morbidity of 60 per cent in Puerto Rico, 48 per cent in New Mexico and 20 per cent in Georgia and an over-all ratio of convalescent or passive carriers to cases of 9.1.

Dysenteric infections seem to be most common in hot countries and in the summer months in temperate climates, although they may occur at any season of the year. The spread of the disease is due to the more or less direct transfer of the specific bacillus from infected intestinal discharges to the alimentary tract of a fresh individual. Polluted water may play a part in some outbreaks but is apparently not nearly so important a factor in dysentery as it is in typhoid fever. Improper disposal of excreta permitting dissemination by flies, and the contamination of food by chronic carriers and convalescents, appear to be the most important factors in the spread of bacillary dysentery. The role of insects, especially flies, is probably an important one and the seasonal incidence of bacillary dysentery is in keeping with this. In the epidemic reported by Kuhns and Anderson infected flies were caught in kitchens and

³⁰ Watt and Hardy: Pub. Health Repts., 1945, 60 261.

operating latrines; similar reports are not common. The decline in diarrhea and enteritis of the last few decades is very likely a reflection of the general improvement in sanitary conditions.

At the present time in temperate climates dysentery flourishes especially in insane asylums and other large institutions, where lack of personal hygiene among the inmates favors the transfer of infection. In the Denmark investigation endemic asylum dysentery was found to be a serious and prevalent disease. Whenever it gains a foothold in these institutions it seems to be kept alive chiefly by chronic carriers and proves an obstinate problem. Weekly bacteriological examination in one institution showed that more than 50 per cent of the dysentery patients continued to excrete dysentery bacilli for long periods—in one case over four and one-half years. Epidemic bacillary dysentery is also a disease of armies in the field, where opportunities for the dissemination of infection are frequently very great and extensive outbreaks are common.

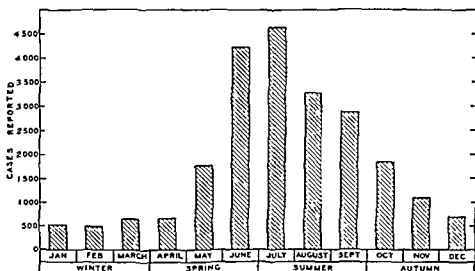


Fig 83 The seasonal incidence of bacillary dysentery. Averages of reported cases by months for the years 1939 to 1942 inclusive. Data from Supplements to Public Health Reports.

Although in small outbreaks a single type of dysentery bacillus may be found, in the larger outbreaks more than one type is almost always observed. The most common types in this country appear to be Flexner, Sonne, Schmitz and *Sh. alkalescens* in that order.

Immunity. Antibodies, agglutinins, are formed in response to infection with dysentery bacilli, usually appearing after the sixth day. The titer is relatively low as a rule. The diagnostic significance of agglutinins is somewhat uncertain largely because "normal" agglutinins are common. Normal serum commonly agglutinates *Sh. shigae* in 1:20 dilution but a titer of 1:40 or higher is suggestive of infection. Agglutinins for *Bact. flexneri* occur in much higher titer, as high as 1:150 in normal serum, and the titer is frequently increased in infections with other species of dysentery bacilli. Flexner agglutinin has, then, very little diagnostic value unless it is to high titer and agglutinins

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for Shiga and typhoid bacilli are absent. Agglutinins to the Newcastle bacillus, *Sh. ambiguum* and *Sh. alkalescens* occur only in low titer. *Sh. sonnei* is often agglutinated to titers as high as 1:50 by normal serum, and, though the titer may occasionally be very high in infection, sometimes little or no agglutinin response is apparent.

Despite the immunological response evidenced by the appearance of agglutinins, there is little or no resistance to second attacks and no method of active immunization, other than to the soluble toxin of *Sh. shigae*, has yet proved effective in bacillary dysentery. In this connection it may be recalled that the infection is confined to the lumen of the intestine, intestinal mucosa

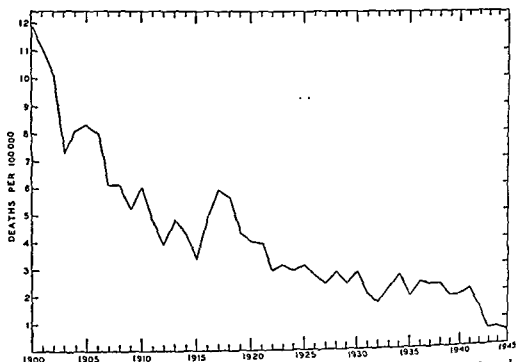


Fig. 84. The prevalence of dysentery in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census

and lymphoid tissue as a rule and in a very real sense remains outside the body. Bacillary dysentery, then, differs from typhoid fever in the important respect that the bacilli do not invade the tissues and consequently are not exposed to the action of circulating antibody and phagocytic cells. Possibly this accounts, at least in part, for consistent failure to develop an effective immunity to infection with these bacilli.

Pathogenicity for Lower Animals. As pointed out above, the cell substance of the dysentery bacillus is markedly toxic to experimental animals upon parenteral inoculation. The Shiga bacillus is by far the most toxic. Considerable quantities of the other dysentery bacilli are required to elicit symptoms of toxemia. Feeding with dysentery bacilli is generally not successful, though the administration of large doses of living bacilli has resulted in dysenteric symptoms in cats and rhesus monkeys. An experimental infection of the urinary bladder of female guinea pigs has been described by Bingel³¹

³¹ Bingel Ztschr. f. Hyg. u. Infektionskr., 1943, 125-110, *ibid.*, 1944, 125-574, 610.

so that in the 1931-5 quinquennium it was only about 15 per cent. of what it was at the close of the last century. Since 1930, however, the rate has remained more or less stable.

TABLE 132

AVERAGE ANNUAL DEATH-RATE FROM DIARRHOEAL DISEASES IN INFANTS UNDER 1 YEAR OF AGE PER 1,000 LIVE BIRTHS. ENGLAND AND WALES.

Years	Average Annual Death Rate	Years	Average Annual Death Rate
1896-1900 . . .	31	1931-35 . . .	5
1901-05 . . .	23	1936-39 . . .	5.1
1906-10 . . .	18	1940-42 . . .	4.8
1911-15 . . .	19	1943-45 . . .	5.0
1916-20 . . .	9	1946-48 . . .	4.1
1921-25 . . .	8	1949 . . .	2.6
1926-30 . . .	6	1951 ¹ . . .	1.2

¹ Figures are in accordance with the International Statistical Classification of Diseases.

If we extend our survey to include young children as well as infants, we see from Table 133 that nearly 90 per cent. of the deaths now occur during the first year of life and over 95 per cent. during the first two years. The footnote suggests that a higher proportion of males die than females.

TABLE 133

DEATHS AT AGES FROM ENTERITIS AND DIARRHOEA. ENGLAND AND WALES.

Year	Age in Years						
	0-	1-	2-	3-	4-	0-5	All Ages
1911	31,899	6,901	1,097	380	185	40,462	46,212
1916	8,313	1,973	438	184	111	11,019	14,702
1921	11,705	1,924	320	154	102	14,205	17,086
1926	5,394	969	201	129	77	6,770	8,415
1931	3,284	447	99	55	48	3,933	5,222
1936	3,220	260	74	37	25	3,616	4,990
1941	2,757	228	68	46	24	3,123	4,633
1946	3,341	133	27	12	6	3,519	4,919
1951 ¹	831	68	26	7	7	939	1,365

¹ Figures are in accordance with the International Statistical Classification of Diseases.
NOTE.—In 1951 59.7 per cent. of the deaths under 5 years of age were in males.

Table 134 records the deaths from diarrhoea and enteritis according to the month of the year. Though deaths at all ages are included, it will be realized from the previous table that most of them refer to deaths during the first two years of life. It is evident that in the hot year of 1921 there was a striking seasonal incidence, the deaths during the months of August, September and October exceeding those during the other nine months of the year. Though detailed figures are not provided in the Registrar-General's report, it is known that in the hot year of 1911, when there was a very high diarrhoeal mortality, the majority of the deaths likewise occurred during the same period. Since 1921, in spite of seven hot years, the gross excess of deaths during the late summer

in which the local pathology was similar to that found in human bacillary dysentery but there was also an invasion of various tissues and organs. Lower animals apparently do not suffer from bacillary dysentery under natural conditions, although it is not uncommon in the laboratory to isolate *Sh. flexneri* from monkeys that develop enteric symptoms.

is often exposed, and to which the devastating outbreaks of summer diarrhoea that occurred in the early years of the present century can be largely ascribed.

Reasons suggested for the beneficial action of breast-feeding are that (a) it lowers the pH of the intestinal contents, which are rendered less favourable for the growth of coliform bacilli; (b) it leads to the production of formic acid, which is present in greater concentration in the faeces of breast-fed infants than of those fed on cows' milk, and which prevents coliform bacilli from multiplying, (c) it provides a growth factor for *Lactobacillus bifidus*, which is partly responsible for the acid reaction of the intestinal contents of breast-fed babies (see Ross and Dawes 1945). The resistance of breast-fed infants appears to last only so long as they are fed entirely on human milk. Complementary feeds of cows' milk or of glucose water lead to an immediate rise in the pH of the intestinal contents and the consequent replacement of a lactobacillary by a coliform flora.

Social and environmental factors play a part in the epidemiology of infantile enteritis. Broadly speaking, the lower the social class, the greater the degree of overcrowding in the homes, and the dirtier the domestic conditions, the commoner is the disease. Poorly nourished babies are more likely to be affected than those which are well nourished. The disease is particularly prevalent in hospitals and institutions, where it constitutes a serious hazard to life. Dobbs (1941), for example, goes so far as to say that gastro-enteritis is the greatest menace to infants suffering from pyloric stenosis admitted to English hospitals. Premature infants nursed in hospitals, particularly if they are not breast-fed, are likewise peculiarly subject to the disease.

Primary Infective Enteritis due to known Pathogenic Organisms

Observations, particularly during recent years, have rendered it evident that some cases of enteritis during infancy and early childhood are due to infection with members of the *Shigella* or *Salmonella* group. How frequent these are, it is impossible to say. In the past many cases have probably not been recognized, owing to imperfect bacteriological technique; but now that increasing attention is being drawn to these organisms, and highly selective media are available for their cultivation, they should be diagnosed more frequently.

Hormaeche (1943) who, with his colleagues, conducted a careful investigation into the enteritis of infancy in Uruguay, found that of 668 cases of enteritis, 260 (38.9 per cent.) were due to *Shigella* and 152 (22.8 per cent.) to *Salmonella* species; in the remaining 256 cases (38.3 per cent.) no pathogenic organisms could be demonstrated (see also Hormaeche *et al.* 1947). The incidence of dysentery and salmonella infections is probably much lower in this country than in Uruguay; but that it is not negligible is shown by the fact that Blacklock and Guthrie (1937), working at the Royal Hospital for Sick Children, Glasgow, investigated during the years 1931-36 no fewer than 215 cases of dysentery in children up to 12 years of age, and Guthrie and Montgomery (1939), at Glasgow, investigated, apparently during the years 1937 and 1938, 28 cases of salmonella infection in infants under two years of age. In Syria, Moore and Dennis (1940) studied 543 attacks of enteritis occurring in 490 infants and children, and found that 65 were due to dysentery bacilli, 11 to proved salmonella strains, and 51 to strains that gave the sugar reactions of salmonella species but were not serologically identified. Hardy and Watt (1948) during a household survey found that 75 per cent. of children dying of diarrhoeal diseases in Georgia and New Mexico were infected with shigellae, in New York City, on the other hand, dysentery appeared to play a very small part in the diarrhoeal death-rate. Sometimes the disease is widespread in the area, as in the outbreak at Brisbane in 1947 caused

THE CHOLERA VIBRIO AND RELATED FORMS

Although Asiatic cholera has doubtless smoldered endemically in parts of India for many centuries, the year 1817 marked its first considerable extension beyond the borders of that country. Europe was first invaded in 1831, and since that date a series of great epidemics has carried the disease over a large part of the civilized world. The disease was brought to New York by Irish immigrants during the pandemic of 1832-33 and in the pandemic of 1846-62 invaded the United States via New Orleans (1848) and spread up the Mississippi Valley. The fourth great pandemic, that of 1864-75, affected Asia, Africa, Europe and America. The causal agent of the disease, the cholera vibrio, was discovered in 1883 by Koch¹ in the intestinal discharges of cholera patients. Similar microorganisms were described by later workers in infected water and elsewhere, and now a number of species are known. In general, however, these other vibrios are nonpathogenic and have been studied in terms of their relation to the cholera vibrio and are, therefore, not particularly well known. Of what is now a fairly sizable group, only two species are pathogenic, the microorganism discovered by Koch and variously termed *Spirillum cholerae asiaticae* (Koch), *Spirillum cholerae*, *Vibrio cholerae*, the comma bacillus, and *Vibrio comma* (Bergey); and a vibrio pathogenic for pigeons and guinea pigs, *Vibrio metchnikovii*.

VIBRIO CHOLERAЕ

Morphology and Staining. The cholera vibrio is a short, slightly curved and twisted rod, 1.5 to 3 μ in length and 0.4 to 0.6 μ in breadth. It may occur singly or in chains which have the appearance of short spirals or S-shaped forms (two cells). The straight and spiral threads formed in the pellicle of liquid gelatin cultures are usually regarded as involution forms. Cultures that have been maintained for a long time on agar often lose the curved form and appear as straight rods, but resume the more characteristic form when passed through animals. The vibrios are actively motile by a single polar flagellum which is shorter than the flagella of most bacteria. Spores are not formed. The cholera vibrio stains readily with the ordinary aniline dyes and is gram-negative.

Colonies on agar media are similar to those of the other enteric bacilli, but may be distinguished from those of *Bact. coli* by their thin, opalescent appearance. They are 1 to 2 mm. in diameter, low, convex and grayish-yellow in color, with a finely granular consistency which is accentuated under low mag-

¹ Koch: Ber. klin. Wehnschr., 1884, 21-477, Brit. Med. Jour., 1884, ii.403, 453.

homes; but it is quite possible that sporadic cases occur which are not recognized as being of the same nature as the epidemic disease. Infants are said to be attacked chiefly in the first fortnight after birth, but again too much weight should not be laid on this statement, because this happens to be the usual length of stay in a maternity home. Infants developing the disease after they have left the home will often be overlooked. Ormiston (1941), describing one outbreak, mentions that when, for various causes, infants were kept in hospital for longer than the usual 10- to 14-day period, 46 per cent. were older than a fortnight when first affected. Under institutional conditions the commonest age at the time of onset is 5 to 8 days. There is no apparent difference in sex incidence; and though infants of the poorer classes are said to be more susceptible than those of the wealthier classes, social distinctions do not appear to be of great importance (Frant and Abramson 1938). Reports on the relative incidence of the disease in breast-fed and artificially fed infants are conflicting (Rice *et al.* 1937, Best 1938, Baker 1939, Forbes and Olsen 1939, Cron *et al.* 1940, Ormiston 1941). Many authors have observed no difference between the two groups; but this may perhaps be partly explained by the common custom in maternity hospitals of giving glucose water or some similar drink to all babies, thus affording a possible means of spreading infection. It is difficult to estimate the true morbidity of this disease.

Rice, Best, Frant and Abramson (1937) say that among 3,672 babies born alive in eleven lying-in institutions in New York City between July, 1934, and December, 1936, 505 are known to have contracted neonatal diarrhoea—a morbidity rate of 14 per cent. Among these the case-fatality rate was 46 per cent. Greenberg and Wronker (1938), referring to a New York hospital, mention a morbidity rate of 40 per cent. and a case-fatality rate of 29 per cent. In Great Britain Ormiston (1941) described outbreaks in three geographically separate institutions, and observed among 996 exposed infants a morbidity rate of 14 per cent. and a case-fatality rate of 29 per cent. In Sakula's (1943) series of 25 cases, no fewer than 20 deaths occurred.

Most authors agree on the high case-fatality rate, which may reach even 80 per cent., but the high morbidity rates just quoted must be understood to refer only to institutions in which the disease occurs. So far as can be ascertained from reported figures, the disease is unknown in most maternity hospitals, so that its total incidence is probably low. There is little to suggest any particular seasonal prevalence; outbreaks occur in the winter as well as the summer. The incubation period may be as short as 2 days or as long as 20 days; the commonest period seems to be 2 to 6 days. In any given nursery there may be two or three sporadic cases, followed by a rapid succession of cases, giving to the outbreak an almost explosive suddenness; or there may be small groups of cases separated by intervals of a week or more.

This is not the place to describe the symptomatology of the disease. Briefly, however, the chief manifestations are a sudden or insidious onset, vomiting, slight fever, diarrhoea, rapid dehydration and loss of weight, often followed by death in 1 to 7 days. Blood, mucus or pus in the stools are uncommon. At autopsy, the findings vary greatly. Often there is little to be seen besides congestion of the intestinal mucosa and slight swelling of the mesenteric lymph nodes. Sometimes there are hæmorrhagic pneumonia and acute hæmorrhagic splenitis (Colvin and Emory 1941); and sometimes cerebral oedema and petechial hæmorrhages in the brain and viscera (Lyon and Folsom 1941, Bloch 1941). Acute enteritis

nification Some strains are hemolytic on blood agar while others are not (see below).

Physiology. The cholera vibrio is strongly aerobic and only very sparse growth appears under anaerobic conditions, and then on prolonged incubation. It grows over the temperature range of 16° to 42° C. with an optimum growth

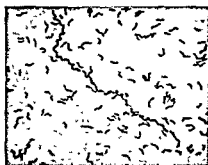


Fig 85 *Vibrio cholerae*, broth culture two days old; fuchsin stain, $\times 1000$ (Frankel and Pfeiffer).

at 37° C. An alkaline reaction is essential for good growth, the bacteria will grow over a pH range of 6.4 to 9.6 and are usually cultivated at an alkaline reaction, pH 7.8 to 8.0. This marked tolerance for alkali is taken advantage of in the preparation of selective media for the isolation of the cholera vibrio, the pH of such media usually being about 9.5. They are not nutritionally fastidious and may be grown in peptone water

THE HEIBERG FERMENTATION TYPES OF VIBRIOS

Sugar	Type					
	I	II	III	IV	V	VI
Sucrose	+	+	+	+	-	-
Mannose	+	-	+	-	+	-
Arabinose	-	-	+	+	-	-

Fermentation reactions are variable and a number of carbohydrates, including dextrose, levulose, galactose, maltose, sucrose and mannitol, may be fermented with the production of acid but no gas. Lactose, inulin and dulcitol are not attacked. Heiberg² has studied the fermentation reactions of the vibrios at some length and has arrived at six fermentative types on the basis of the fermentation of sucrose, arabinose and mannose which are known as the Heiberg types. Type I is characterized by the fermentation of sucrose and mannose but not arabinose, and contains all of the cholera vibrios and some non-cholera

² Heiberg, C. R. Soc. Biol., 1934, 115:984

of *Bact. coli* isolated in infantile gastro-enteritis is increasing gradually; besides O 55 and O 111, Types O 26, O 86 and O 119 are now recognized.

It should be pointed out that the presence of these organisms is not always associated with epidemic disease. Thus Payne and Cook (1950) isolated Type O 111 from a number of healthy babies in a residential home in Oxfordshire; at one time 10 out of the 15 infants in the home were excreting this organism in the absence of any outbreak of gastro-enteritis. Likewise, Cathie and MacFarlane (1951) at the Great Ormond Street Children's Hospital in London found that 17 out of 85 infants under 2 years of age admitted to the hospital for some disease other than gastro-enteritis were excreting Type O 111. This, however, is not surprising. Practically every pathogenic bacterium known to cause an epidemic disease is also found associated with endemic infections of the same kind.

The pathogenicity of certain specific types of *Bact. coli* is supported by observations on human volunteers in the United States of America.

Neter and Shumway (1950), for example, gave 100 million viable organisms of Type O 111 by the mouth to a 2-month-old mentally deficient infant. Severe diarrhoea followed and the infant lost 7 ounces in weight in 24 hours. The organisms were abundant in the stools, but disappeared within 2 days under terramycin treatment. Previous administration of a different type of *Bact. coli* had been without effect. Ferguson and June (1952) found that 23 volunteers given 6,000 million to 9,000 million organisms of Type O 111 by the mouth all developed gastro-enteritis about 10 hours later, whereas a strain of *Bact. coli* of a different serological type isolated from a healthy infant had no effect on 11 volunteers. Later June, Ferguson and Worfel (1953) made similar observations on the pathogenicity of Type O 55, though the disease produced by this organism was milder than that caused by Type O 111. Generally speaking with both organisms the severity of the symptoms corresponded with the size of the dose used.

On the whole, the evidence is becoming increasingly stronger that certain specific types of *Bact. coli* are intimately concerned with the production of gastro-enteritis both in the new-born and in older infants, and there is every reason to believe that further investigation will strengthen this provisional conclusion.

Many workers favour a virus as the primary cause of infection. Little evidence, however, has so far been brought to support this view; and the fact that, apart from the virus diarrhoea of suckling mice (Cheever and Mueller 1947), diseases of the intestinal tract caused by viruses are almost unknown, indicates the desirability of caution in accepting it. Light and Hodes (1943, 1949), it is true, claimed to have demonstrated the presence of a filtrable agent in the stools of cases of neonatal diarrhoea capable of giving rise to acute enteritis in calves, but the human origin of the virus they studied is doubtful. The positive results on medical students recorded by Reimann, Price and Hodges (1945) are open to the objection that they were obtained during a period when diarrhoea, nausea and vomiting was epidemic in the area and no precautions were apparently taken to segregate the students during the tests. More convincing evidence was brought by Jordan, Gordon and Dorrance (1953), who carried out a number of experiments on young adult volunteers. They noted the existence of two different clinical forms of infantile gastro-enteritis. (a) one causing an afebrile illness, with an incubation period of about 60 hours and a duration of 4 days, and characterized by initial vomiting, diarrhoea with frequent watery stools, little constitutional disturbance, and a tendency to spread to other infants; and (b) one causing a febrile illness, with an incubation period of 27 hours and a duration of only 1 day, and characterized by initial vomit-

varieties. Starch is hydrolyzed. Both coagulated serum and gelatin are liquefied. Stab cultures in gelatin often develop a small turnip-shaped area of liquefaction at the surface, which by evaporation of the fluid leaves a bubble-like depression, while in some growth little or no liquefaction occurs along the needle puncture. Other vibrios besides the cholera vibrio, however, produce this same type of liquefaction. Growth in milk does not produce any visible change for some time, but a slow peptonization without coagulation appears on continued incubation. Hydrogen sulfide and indol are produced and nitrates are reduced to nitrites. The addition of sulfuric acid to a culture of the cholera vibrio in nitrate-peptone broth results in the development of a red color—the so-called “cholera-red reaction.” This is, of course, the nitroso-indol reaction and is given by any bacterium, as the colon bacillus for example, that both reduces nitrate and produces indol. Other vibrios give this reaction also.

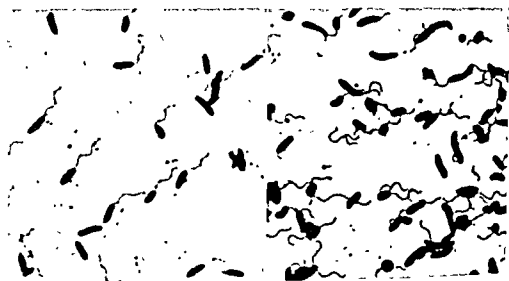


Fig 86. *Vibrio cholerae* (left) and the vibrio of Finkel and Prior (right), showing the single polar flagella. $\times 2000$ (Kral).

The question of the hemolytic activity of the cholera vibrio has been of considerable importance in relation to its differentiation from the closely related El Tor vibrios. The term hemolysis, when applied to the vibrios, refers to hemolytic activity as determined by the Greig test in which a 3 per cent suspension of goat erythrocytes is mixed with an equal quantity of twenty-four-hour broth culture of the vibrio and read after two and four hours' incubation at 37°C . By this test the cholera vibrio is non-hemolytic.³ As indicated above, however, some strains of the cholera vibrio are hemolytic on blood agar. The apparent contradiction has been resolved by van Loghem⁴ in particular, who has shown that in the case of this bacterium at least, blood plate hemolysis is a hemodigestive process and basically different from the liberation of hemoglobin from suspensions of erythrocytes.

The resistance of the cholera vibrio to various injurious influences is not great. It is killed by moderately high temperatures (ten minutes at 55°C .) and is destroyed quickly by chemical disinfectants. It is particularly sensitive

³ For a recent study see Read, Pandit and Das. Indian Jour. Med Res., 1942, 30 183

⁴ Cf. van Loghem. Centralbl. f. Bakt., I Abt., Orig., 1913, 67 410.

promotes the multiplication of bacteria in milk and similar foods. This factor will clearly be operative to a greater extent in poor-class houses, ill provided with the means of keeping foodstuffs clean and cool, than in the houses of the well-to-do.

The bacteriological findings in summer diarrhoea have been most diverse. They were reviewed in some detail in the 2nd edition of this book (pp. 1250-53). Suffice it to say here that members of the dysentery group, *Proteus morgani*, *Proteus vulgaris*, *Ps. pyocyanea*, *Cl. welchii*, and certain other organisms were all incriminated by workers in different countries; but that no one single pathogenic organism was demonstrated by everybody. The only common finding was that non-lactose-fermenting organisms were greatly increased in the faeces; what the majority of these organisms really were remains in doubt. The bacteriological investigation of summer diarrhoea was carried out at a time when our technical methods were far less developed than they are now, and when many members of the *Shigella* and *Salmonella* groups were unknown. It is fairly clear that no one organism could have been responsible for all cases. We are therefore driven to the conclusion that summer diarrhoea, though appearing epidemiologically to be a well-defined disease, was in fact associated with a multiplicity of bacterial agents. On the whole it seems probable that the disease was a bacterial infection, the prevalence of which was determined by a number of factors, varying to some extent independently, but all conditioned directly or indirectly by the prevailing temperature.

During the past 20 or 30 years, summer diarrhoea has practically disappeared from this country (see Table 134). The better disposal of garbage, the diminution of dust, the replacement of horse by motor traffic, the decrease in the number of flies, the improvement in cleanliness of the milk supply, and the greatly increased use of pasteurized, boiled and dried milk in the feeding of infants and children, have probably all aided in lessening the chances of bacterial contamination of food with pathogenic bacteria.

Enteritis following the Consumption of Food containing Toxic Substances of Bacterial Origin

Little is known of this group. It is included in Lyon's (1940) classification of the diarrhoeal diseases of children; but owing to the peculiar difficulty of demonstrating bacterial toxins concerned in food poisoning, other than that formed by *Cl. botulinum*, progress in its study has necessarily been slow. Some strains of staphylococci are known to give rise under favourable conditions to an enterotoxin capable of causing acute gastro-enteritis in adults. The toxin may be formed in milk, and it is therefore almost certain that infants and young children fed on raw cows' milk must be affected from time to time. Whether other common organisms proliferating to a prodigious degree in milk can render the milk toxic to infants, either by virtue of their own body substance or by breaking down some substrate in the milk, is still doubtful. Many years ago Park and Holt (1903) brought evidence to show that milk containing large numbers of coliform bacilli, streptococci and micrococci, i.e. bacteriologically dirty milk, was able to give rise to gastro-intestinal disturbance in infants, but the exact mechanism by which this occurs has so far baffled inquiry (see Chapter 93).

Infective Enteritis of Protozoal Origin

There is considerable reason for believing that the flagellate, *Giardia lamblia*, may give rise to enteritis in infants and young children. This organism hooks

to drying, if a drop of broth culture be dried on a slide, the vibrios are all dead in about two hours. It does not survive long in association with the ordinary saprophytic bacilli of soil and water, and whether it is able to multiply outside the body in impure water is uncertain. Upon the surface of vegetables and fruits kept in a cool, moist place, the vibrios may remain viable for from four to seven days. The slight resistance of the cholera vibrio, especially its sensitiveness to drying, explains the rapid and complete disappearance of cholera in once infected localities, and also the circumstance that the disease is rarely, if ever, air-borne.

The biochemical structure of the vibrios has been studied at some length by Linton and his co-workers.⁵ They have described three types of polysaccharide and two types of protein present in the cholera and related vibrios. The poly-



saccharides were differentiated on the basis of their hydrolysis products; that designated type I was made up of galactose and an aldobionic acid (galactose and glucuronic acid), type II of arabinose and aldobionic acid, and type III was a polymer of glucose. The two protein types, both globulins, were differentiated on the basis of racemization, i.e., optical activity of the hydrolyzed protein before and after alkali racemization. These substances occurred in combinations to give six groups of vibrios, the cholera vibrios falling into groups I and II which contained the same protein but polysaccharide types I and II respectively. The significance of these observations is by no means clear. It is evident, however, that the polysaccharide bears no relation to the immunological character of the vibrios for the cholera vibrios are immunologically homogeneous (see below), and it is inconceivable that galactose- and arabinose-containing polysaccharides should show identical immunological specificity. Apparently neither the protein nor polysaccharide fractions are associated with the endotoxin.

⁵ Summarized in the review by Linton: *Bact. Rev.*, 1940, 4:261.

it is well to remember that large numbers of children who suffer from respiratory infection do not develop gastro-enteritis, and that often quite a high proportion of cases of true dysentery develop parenteral infection (Marriott *et al.* 1933). Respiratory infections are much commoner in the winter than the summer, whereas gastro-enteritis tends, if anything, to show the reverse seasonal incidence. It is improbable, therefore, that parenteral infection is responsible for more than a small proportion of cases of gastro-enteritis. This conclusion is also reached by Wright and Wright (1946) in a paper which, in its statistical approach to the problem of infantile diarrhoea and enteritis, well repays study.

We may perhaps include here a mention of occasional outbreaks of infantile enteritis that accompany an influenzal type of disease in the mothers and nursing staff; the disease in the babies has a high case-fatality rate, and hæmorrhagic pneumonia may be found *post mortem* (Kirby *et al.* 1950).

In the United States Buddingh and Dodd (1944) described a disease of infants characterized by stomatitis and to a less extent diarrhoea. Numerous fine vesicles appear on the mucosa of the tongue and gums and go on to superficial ulceration and desquamation. There is little or no fever. In about a third of the cases there is an accompanying diarrhoea with much mucus and occasionally flecks of blood. The disease lasts for 7-10 days, but often relapses. It is highly contagious. Evidence is brought to show that the causal agent is a filtrable virus which is present in the vesicles and in the stools. Inoculated on to the scarified cornea of the rabbit it gives rise to a severe inflammatory reaction which passes off in 3-4 days and which unlike herpes is not purulent, does not leave behind a permanent corneal opacity, and is not characterized by the presence of inclusion bodies.

Prophylaxis and Treatment

Broadly speaking, the prevention of enteritis lies in the protection of the food supply from contamination, the provision of good general hygienic conditions, and the separation as far as possible from each other of infants and children under 2 years of age. Reference has already been made under summer diarrhoea to the beneficial effect of reducing the fly population (see also Lindsay *et al.* 1953), and to the replacement of raw by pasteurized, boiled or dried milk. Whenever possible, breast-feeding should be preferred to artificial feeding. Raw cows' milk may be contaminated from so many different sources—the cow itself, the person handling the milk, impure water used for cleansing the utensils, dirty bottles, flies, and rodents—that it should never be given to infants and young children. Even milk produced under cleanly conditions on the farm, such as Tuberculin Tested milk, may be contaminated from one or more of the sources just mentioned, and should therefore always be pasteurized or brought rapidly to the boil and cooled.

The importance of good hygienic conditions is obvious. In particular, care should be exercised to protect infants against exposure to undue heat or cold, and to minimize their risk of contact with other persons suffering from respiratory or intestinal infection. The desirability of rearing infants during their first two years of life under relatively segregated conditions is not yet fully appreciated. Infantile diarrhoea is so common in institutions that no encouragement should be given to the establishment of nurseries for children under 2 years of age. No infant should be
 be carried out effectively
 ers of infants die unneces-
 sarily every year as the result of contracting gastro-enteritis in hospitals to which

Toxin. The evidences of profound toxemia in human infections with the cholera vibrio are strongly suggestive of the formation of toxic substances by this microorganism. It was early shown by Pfeiffer⁶ that, while filtrates of young broth cultures were only slightly toxic, vibrios killed with heat or chloroform from young agar cultures were markedly toxic to guinea pigs on intraperitoneal inoculation. The toxicity of cell-free filtrates of old broth cultures led a number of the earlier investigators to the conclusion that a soluble exotoxin is produced. It is now established, however, that no exotoxin is formed and the toxicity of old broth cultures is attributable to dissolution of the vibrios and the liberation of endotoxin.

The endotoxin is heat-stable and it was early found by Metchnikoff to be diffusible through collodion sacs.⁷ The isolation of a toxic protein has been reported by some workers⁸ and the toxicity has been extracted by the trichloroacetic acid method.⁹ On the basis of the latter observations it has been assumed that the toxin is a polysaccharide-lipid complex similar to those isolated from typhoid and dysentery bacilli; this conclusion is not, however, supported by unequivocal evidence. More recently it has been found¹⁰ that the cholera toxin is associated, and possibly identical, with a dialyzable phospholipid which may be extracted from the intact vibrios with organic solvents such as alcohol, ether and chloroform.

Though in general the pharmacological activity of the bacterial endotoxins is not characteristic, it has been reported¹¹ that the intravenous inoculation of experimental animals with toxic preparations produces a diarrhea and pathologic changes in the intestine and kidneys not unlike those observed in human cholera. It has been shown by Burrows, Wagner and Mather¹² that purified toxin markedly increases the permeability of the isolated strip of intestine to fluids. In any case, the cholera toxin seems to have a special affinity for the epithelium and causes a shredding of the epithelium of the intestine and gallbladder in human cholera.

The antigenicity of the cholera toxin has been open to some question for, in general, the antitoxic qualities of cholera antiserum are only feeble at best. Some workers have, however, reported¹³ the preparation of effective antitoxin. The purified lipid toxin has been found to be antigenic in that antisera to it

⁶ Pfeiffer. *Ztschr. f. Hyg.*, 1892, 11:393, Pfeiffer and Wassermann. *ibid.*, 1893, 14 46.

⁷ For more recent studies see Basu, Chaudhury and Basu: *Calcutta Med. Jour.*, 1940, 36:571, and Banerjee: *Jour. Indian Med. Assn.*, 1942, 11 95.

⁸ Galeotti. *Centralbl. f. Bakt., I, Orig.*, 1912, 67:225; Sanarelli: *Ann. Inst. Pasteur.*, 1920, 34:370, Hahn and Hirsch. *Centralbl. f. Bakt., I, Orig.*, 1927, 104:211; *ibid.*, *Klin. Woch.*, 1927, 6:312, *ibid.*, *Ztschr. f. Hyg. u. Infektionskr.*, 1929, 110:355.

⁹ Checcacci. *Boll. Inst. Sieroterap. Milanese*, 1939, 18:391, Raynal, Lieou and Feissolle. *Rev. Immunol.*, 1939, 5 317, *ibid.*, 1940, 6:132, Damboviceanu and Barber: *C. R. Soc. Biol.*, 1940, 133:501, Gallut. *Ann. Inst. Pasteur*, 1943, 69 123.

¹⁰ Burrows. *Proc. Soc. Exp. Biol. Med.*, 1944, 57:306.

¹¹ Hahn and Hirsch. *Klin. Woch.*, 1928, 7:2483, Gosh: *C. R. Soc. Biol.*, 1933, 112 1176; Pham. *ibid.*, 1935, 119:78.

¹² Burrows, Wagner and Mather: *Proc. Soc. Exp. Biol. Med.*, 1944, 57 311

¹³ Pottevin. *Bull. Soc. Path. Exot.*, 1913, 6:409, Horowitz. *Ztschr. f. Immunitätsf.*, 1913, 19 44, Bail. *ibid.*, 1916, 25 248, *ibid.*, 1917, 26 97, Hahn and Hirsch: *Centralbl. f. Bakt., I, Orig.*, 1927, 104 211, *ibid.*, *Ztschr. f. Hyg. u. Infektionskr.*, 1929, 110:355, Andu and van Niekerk. *Centralbl. f. Bakt., I, Orig.*, 1929, 112 519.

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show marked protective qualities as measured by the mouse protection test.¹⁴

Antigenic Structure. The first demonstration of bacterial agglutination with homologous immune serum by Gruber and Durham¹⁵ was the agglutination of the cholera vibrio and the typhoid bacillus. It was soon apparent that the agglutination reaction was specific and it has been used for the identification of the cholera vibrio since. The antigenic structure of the cholera and related vibrios has, therefore, been of considerable interest in connection with the differentiation of *V. cholerae*.

The existence of serologic types within this group was shown by Japanese workers Kabeshima¹⁶ demonstrated the existence of two types by cross absorption and designated one "original" or J, and the other "variant" or F. A third serologic type was found by Nobechi¹⁷ which he designated "intermediate" or "middle" since it appeared to have affinities with the other two. The "original" and "variant" types, sometimes called "end types," are now known as Inaba and Ogawa types respectively, and the "middle" type is called Hikojima. All three are equally capable of causing epidemic cholera, but Inaba is most common, practically the only type in India, and Hikojima is least common.

It was shown by Balteanu¹⁸ that vibrios, like other motile bacteria, contain both heat-stable, somatic, or O antigen and heat-labile flagellar or H antigen. Shousha¹⁹ found that the O antigen of the cholera vibrio is specific, while the H antigen is shared with a variety of non-cholera vibrios such as water vibrios and the like. Gardner and Venkatraman²⁰ have carried this analysis further and found that most vibrios can be differentiated into six groups which they designated by Roman numerals based on the specificity of the O antigen. Group I contains all of the cholera vibrios and some El Tor vibrios. Serological identification of the cholera vibrio, therefore, requires the use of specific O antiserum. Since the El Tor vibrios are hemolytic, the cholera vibrio is defined as a non-hemolytic member of Group I. Gardner and Venkatraman account for the specific agglutination of earlier workers on the basis that O agglutination occurs more rapidly than H agglutination and hence was observed first. A detailed analysis of the O and H antigenic structure of the cholera and related vibrios by Burrows *et al.*²¹ showed that a group-specific O antigen, designated A, is shared by all vibrios of Group I, and that the Japanese types are determined by subsidiary O antigens, B and C, and arbitrarily designated type-specific. It was suggested that the Japanese types be indicated by their antigenic formulae, i.e., Inaba as Type AC, Ogawa as Type AB, and Hikojima as Type ABC. Vibrios containing the group antigen but lacking these type-specific antigens have been found and thus constitute a new type. The H antigens, designated by arabic numerals, and other components of the O antigen are shared by the O Group I vibrios with vibrios of other O groups.

¹⁴ Burrows, Mather, Wagner and McGann. *Proc. Soc. Exp. Biol. Med.*, 1944, 57 308.

¹⁵ Gruber and Durham. *Muench. Med. Woch.*, 1896, 43 285.

¹⁶ Kabeshima as early as 1912, original articles in Japanese, cited by Nobechi.

¹⁷ Nobechi. *Sci. Rept. Govt. Inst. Inf. Dis.*, Tokyo, 1923, 2 29, 43.

¹⁸ Balteanu. *Jour. Path. Bact.*, 1926, 29 251.

¹⁹ Shousha. *Jour. Egypt. Med. Assn.*, 1931, p. 438.

²⁰ Gardner and Venkatraman. *Jour. Hyg.*, 1935, 35 262.

²¹ Burrows, Mather, McGann and Wagner. *Jour. Inf. Dis.*, 1946, 79 168.

CHAPTER 72

BACTERIAL FOOD POISONING AND BOTULISM

FOOD POISONING

Definition.—The consumption of unwholesome food or drink may give rise not only to well-known bacterial infections such as enteric fever, dysentery, cholera, tuberculosis, undulant fever, scarlet fever, septic sore throat, and so on, and to helminth infections such as trichinosis, in which the food serves as a vehicle for the specific infective organism, but also to such diverse conditions as those due to the ingestion of inherently poisonous substances—for example deadly nightshade, hemlock, poisonous fish, toadstools, and ergot-infected grain—and those in which the food has become contaminated directly with such poisons as arsenic, tin, antimony, cadmium or other heavy metals, or with chemicals such as sodium fluoride, or tri-(ortho-cresyl)-phosphate, which was responsible for outbreaks of so-called “jake” or “ginger” paralysis in the United States (see Smith and Elvove 1931) and for a similar outbreak in Durban (Sampson 1942). The term “food poisoning,” however, is conventionally restricted to acute gastro-enteritis due to the bacterial infection of food or drink. So defined, it is divided into two groups: (1) that following the multiplication within the body of pathogenic organisms contained in the food; this may be referred to as the “infection” type, (2) that following the ingestion of food in which poisonous substances have been formed as the result of bacterial proliferation; this may be referred to as the “toxin” type.

Botulism, which is also a consequence of bacterial proliferation in food, differs from either of the types just described in the character of the symptoms and in the nature of the toxin concerned; though, for convenience, it will be discussed in this chapter in a separate section (p. 1816). Those interested in chemical food poisoning may be referred to the works of Jordan (1931), Dack (1919), and Tanner and Tanner (1953). For a review of food poisoning, see Meyer (1953).

It will be noted that the definition given above excludes food idiosyncrasy and food allergy—conditions in which the food itself is wholesome but the patient's reaction is abnormal. In addition, it excludes acute gastro-enteritis, such as is characteristic of the winter vomiting disease (see p. 2011), in which the infection does not seem to be food-borne; and gastro-enteritis associated with malnutrition and starvation (see García and Covián 1942).

Symptomatology.

In the *infection* type of food poisoning, after an incubation period varying from 4 to 36 hours, but generally between 8 and 24 hours, the illness commences with severe headache, followed by nausea, vomiting, diarrhoea and abdominal pain.

A curious terminology has crept into the cholera literature with respect to agglutination; it is said that a strain which is agglutinated by specific antiserum is "agglutinable" while those vibrios which are not agglutinated by cholera antiserum are called "inagglutinable" despite the fact that they are readily agglutinated by homologous antiserum.

SEROLOGICAL TYPES OF CHOLERA AND PARACHOLERA VIBRIOS

Japanese Type		Immunological Type
Name	Synonyms	
None Inaba Ogawa Hikojima	None "J," Japonica 1911, "original," "end type" "F," Formosicana 1911, "variant," "end type" "Middle type"	Type A Type AC Type AB Type ABC

Variation. The cholera vibrio is well known for its tendency to develop bizarre involution forms. These are found not only in old cultures and cultures grown under somewhat adverse conditions, such as increased salt concentrations, but also are produced by the inclusion of substances such as glycine or alanine in the medium. Such changes in morphology are also associated with the usual type of S-R dissociation which occurs readily under the influence of bacteriophage, lithium chloride, etc., the rough variants showing distinctive colonial character, spontaneous agglutination in salt solution, etc.

The immunological changes associated with the S-R dissociation have been studied in some detail by White.²² He has found that four groups of rough variants are immunologically distinguishable, and his Group A appears to correspond to the Group I of Gardner and Venkatraman. Further degeneration to the so-called ρ variants results in a loss of specific O antigen. An independent type of variation, rugose-non-rugose, may be induced, and the rugose strains of S, R and ρ variants contain an O antigen that is common to both members of Group A and vibrios of other groups.²³ A somatic protein antigen has been isolated by White²⁴ which is common to all known variants and shows wide cross-precipitin reactions throughout the vibrio group, although it appears not to be concerned in the agglutination reaction.

Pathogenicity for Man. The causal connection between Asiatic cholera and the microorganism discovered by Koch has been demonstrated by a number of laboratory accidents. One of the first occurred in Koch's laboratory, and other infections resulting from the accidental swallowing of cultures of the cholera vibrio have been noted since. In one instance the swallowing was deliberate, Pettenkofer and Emmerich voluntarily swallowed a small quantity of broth culture of "Koch's vibrio" and as a result developed cholera.

²² White. Jour. Hyg., 1935, 35:347.

²³ White. Jour. Path. Bact., 1940, 50:160.

²⁴ *Ibid.*, 1940, 50:165.

outbreaks they investigated occurred between May and October. During the years 1941-50, 3,789 (43.2 per cent.) of incidents in England and Wales out of a total of 8,780 about which information was available occurred during the third

TABLE 136

BACTERIAL FOOD POISONING IN ENGLAND AND WALES.

(From records of Ministry of Health and Public Health Laboratory Service.)

Year	Average Annual No of Incidents	Presumed Causal Agents			
		Salmonellæ	Staphylococci	Other Organisms	Unknown
1931-5 . . .	59	32	21	6	
1936-40. . .	81	38	8	7	33
1941-5 . . .	324	270	15	3	60
1946. . . .	684	660	7	8	9
1947. . . .	845	794	20	17	14
1948. . . .	803	749	19	13	22
1949. . . .	2,424	1,369	97	84	874
1950. . . .	3,977	2,021	82	67	1,807
1951. . . .	3,345	1,668	65	25	1,587
1952. . . .	3,518	2,098	82	37	1,301

The term "Incidents" includes outbreaks and sporadic cases, each presumably due to a different source of infection; it does not refer to the total number of persons affected, which is of course much larger because in some outbreaks scores or hundreds of persons were affected. Outbreaks and cases caused by dysentery bacilli are excluded.

Of the 2,021 incidents in 1950 caused by salmonellæ, 467 occurred as outbreaks and 1,554 as sporadic cases.

Of the 82 incidents in 1950 caused by staphylococci, 67 occurred as outbreaks and 15 as sporadic cases.

Of the 37 incidents in 1952 caused by other organisms, 20 were due to *Cl. welchii*.

quarter of the year (Report 1950a, 1951a). Much of our food becomes contaminated in one way or another with potentially pathogenic organisms, but so long as these are not allowed to grow to any extent, the risk of food poisoning is small.

TABLE 137

SEASONAL INCIDENCE OF BACTERIAL FOOD POISONING.

(ENGLAND AND WALES, 1949-1952.)

Quarter	Total	Presumed Causal Agents					Percentage of Total
		<i>Salmonella typhimurium</i>	Other Salmonellæ	Staphylococci	Other Organisms	Unknown	
First . . .	1,308	519	214	32	23	520	12.3
Second . .	2,313	1,150	399	64	30	669	21.8
Third . . .	4,500	2,492	686	172	78	1,071	42.3
Fourth . .	2,507	1,132	350	35	82	907	23.6
Not stated	2,643	118	96	23	4	2,402	—
Total . .	13,271	5,411	1,745	326	217	5,569	

Table 137 shows that all classes of bacterial food poisoning are most frequent in the summer months and not only those due to the less specific organisms, indicat-

Both laboratory cases of cholera and those cases contracted naturally in the course of epidemics are marked by great differences in the susceptibility of different individuals. This is probably a consequence in part of innate individual differences in natural resistance, and in part to predisposing factors. Fatigue, the excessive use of alcohol, and various factors leading to mild, non-specific gastro-intestinal derangements predispose in a marked degree to attacks of cholera.

The incubation period is short, it is usually given as three to five days but may occasionally be as short as twenty-four hours. When the vibrios have entered the small intestine and established themselves they multiply rapidly and, with autolysis and dissolution of the cells, endotoxin is liberated and symptoms appear. There are two clinical stages, the first that of *vomiting* and *profuse diarrhea* with the characteristic rice-water stools containing flakes of mucus, shed epithelial cells and enormous numbers of the vibrios. With the tremendous loss of fluid dehydration and hypochloremia become marked, and collapse, the *algid stage*, ensues with circulatory failure, subnormal temperature and anuria. Death may occur in either the first or second stage. The case fatality rate is over 50 per cent in untreated cases and about 30 per cent in treated cases.

At autopsy the small intestine shows marked destruction of the epithelial lining and a characteristic subepithelial edema. It is generally said that ulceration does not occur in cholera, but Goodpasture²⁵ has shown that desquamation of the epithelium is followed by ulceration if the patient survives long enough. Other changes are not characteristic and include a prominence of lymphoid tissue and cloudy swelling of the kidneys.²⁶

Cholera in man appears to be largely, if not entirely, a toxemia, for the infection is confined to the lumen of the intestine and vibrios are found in the organs only rarely. It is not altogether clear whether toxin is absorbed. In the opinion of many the profound dehydration and hypochloremia and consequent impairment of renal function are sufficient to cause the clinical symptoms and pathology.

Carriers. On recovery the vibrios disappear rapidly and are discharged for only a short time. In the series of 200 cases studied by Ying²⁷ 98 per cent were negative by the end of the second week, only a few giving positive cultures as late as the third and fourth weeks. There appears to be no chronic carrier state established in cholera. The statements in the literature regarding chronic carriers refer to persons who discharge "agglutinable" vibrios of the El Tor type, a chronic carrier of typical virulent cholera vibrios has never been observed.²⁸ It is probable that casual carriers occur during epidemics but in no instance has this been demonstrated bacteriologically. While the convalescent carrier, and possibly the casual carrier, may play some part in the spread of the disease, the case, especially in the incubation period, is probably by far the most important source of infection.

²⁵ Goodpasture. Philippine Jour. Sci., 1923, 22 413.

²⁶ The pathology is summarized briefly by Banerjee. Jour. Indian Med. Assn., 1939, 8 39.

²⁷ Ying. Chinese Med. Jour., 1940, 58 595.

²⁸ The carrier question is discussed by Coury. Bull. Office Internat. d'Hyg. Publique, 1933, 25.1149.

and had formed thermostable toxic substances. The subsequent cooking to which the food was exposed destroyed the organisms themselves, but did not seriously affect their toxic products, which were therefore able to give rise to food poisoning on ingestion. No adequate confirmatory evidence of the formation of specific exotoxins by members of the *Salmonella* group was forthcoming, and the balance of evidence appeared to be against this view (but see p. 1812).

While bacteriologists were speculating about the causation of the non-salmonella food-poisoning outbreaks, Dack, Jordan, and their colleagues in Chicago (see p. 1808) drew attention to certain outbreaks of poisoning which were proved to be due to the presence in the food of a toxin formed by staphylococci. By observations made on human volunteers, it was shown that certain strains of staphylococci were able to form toxic substances endowed with some degree of thermostability. These findings were of special interest in that they afforded conclusive proof of the formation of bacterial toxins in the food prior to its consumption. Hitherto the only known instance of this was that of botulism—a disease to be described later, in which the anaerobic bacillus, *Cl. botulinum*, gives rise to an extremely potent specific exotoxin with an action, not on the gastro-intestinal mucosa, but on the nervous system.

Other observations by Jordan and by various continental workers cast serious suspicion on food in which there had been inordinate bacterial multiplication of any type. The inference to be drawn from these observations was that many organisms, non-pathogenic in themselves, such as *Bact. coli*, *Proteus vulgaris*, *Proteus morgani*, milk streptococci and others, when allowed to multiply under favourable conditions in suitable types of foodstuffs, could give rise to toxic substances having an irritating effect on the human gastro-intestinal mucosa. Many workers had toyed with this idea of non-specific toxin formation, but had abandoned it in the absence of adequate evidence. The epidemiological, and more recently the bacteriological, evidence seems to us, however, to be becoming increasingly strong in favour of this view. The pendulum is in fact slowly swinging in the direction of the old "ptomaine" theory, with the distinction that the poisons now regarded as responsible result not from advanced protein decomposition, but from the growth of types of bacteria which can proliferate enormously without greatly altering the appearance and taste of the food. What the nature of these products is remains in doubt. The suggestion that they consist of the autolysed bacterial bodies themselves (see Jordan 1931, Jordan and Burrows 1935, Savage 1933, Report 1934) is not borne out by human volunteer experiments, and it seems more probable that they consist of some breakdown product formed from the food, or of a bacterial enzyme which has a specific affinity for some constituent of the human gastro-intestinal tract.

The real distribution of the different forms of bacterial food poisoning cannot be determined with certainty, since the proportion of more severe cases—mainly of salmonella origin—coming to the notice of the public health authorities is greater than that of the milder cases. Reference to Table 136 shows that in England and Wales the proportion of incidents due to other or unknown organisms rose rapidly after food poisoning was made a notifiable disease in 1949. In 1950 it will be observed that salmonella infections accounted for 50·8 per cent, staphylococcal intoxication for 2·1 per cent, and other or unknown organisms for 47·1 per cent. of the incidents. These figures are very different from those quoted by Feig (1950) for the United States where, of 476 outbreaks in which the bacterial cause

Bacteriological Diagnosis of Asiatic Cholera. As indicated above, the vibrios are present in the ricewater stools in very large numbers and can usually be isolated from fresh specimens *without difficulty*. At times they are present in sufficient numbers that they can be found in stained smears, preferably made from a flake of mucus. Both enrichment and direct streaking of agar media are used for cultures. For enrichment a few drops of the stool are added to a tube of alkaline (pH 8.0 to 9.0) peptone water. The vibrios grow much more rapidly than the other intestinal bacteria and after six to eight hours' incubation form a thin film of growth on the surface of the medium which can be smeared and stained, and streaked on agar.

A number of agar media have been used for isolation of the cholera vibrio, including starch-phenolphthalein agar and the alkaline blood medium of Dieudonné. The latter has been the most generally used. In its preparation an excess of alkali is added and the poured plate must be ripened, i.e., left partly open in the incubator for twenty-four hours to allow the evolved ammonia to escape. With storage the pH continues to drop and the medium becomes less selective and unsatisfactory. The alkaline hemoglobin medium of Vedder and Van Dam²⁹ is buffered with glycine, requires no ripening, and the pH remains constant at 9.5 with storage. It possesses all the advantages and none of the disadvantages of Dieudonné's medium. It is sometimes implied that these media with a pH at the upper limit of tolerance of the cholera vibrio are so highly selective that few if any other intestinal bacteria will grow on them. This is not true, for micrococci may be found in abundance on such plates, the writer has observed counts of 1000 million bacteria per gram of normal guinea pig feces on both Dieudonné's and Vedder and Van Dam's media. However, colonies of the cholera vibrio are readily distinguished and may be picked and identified by slide agglutination with monospecific O anti-serum. With clinically typical cholera in an epidemic this is sufficient identification. A negative Greig test, fermentation of sucrose and mannose and failure to ferment arabinose, and positive indol and nitrite (cholera-red) reactions further substantiate the identification.

Epidemiology. As indicated earlier, the great endemic focus of Asiatic cholera is in India, especially in the delta of the Ganges River. Recent evidence has indicated that there is also an endemic area in China, in the valley of the Yuan River which flows into the Yangtse River through Tun Ting Lake.³⁰ Whether this is recently established or recently discovered is not known. In any case, the disease spreads out in epidemic form from these foci each year, occurring regularly throughout India, in the Yangtse Valley and along the China coast. The entire Far East is affected at one time or another, especially China and French Indo-China, and the disease appears from time to time in Manchuria and Japan. The last general outbreak in the Philippine Islands was in 1934; a few cases appeared in 1935 and 1936. The geographic distribution differs greatly from year to year and that indicated in Fig. 88 is only an approximation.

Infection spreads to the west especially via the pilgrimages, pilgrims going from the delta of the Ganges to Arabia and Mecca transmit it to pilgrims from

²⁹ Vedder and Van Dam: *Nederl. Tijdschr. v. Hyg. Microbiol. en Serol.*, 1932, 7:197.

³⁰ Robertson and Pollitzer: *Trans. Roy. Soc. Trop. Med. Hyg.*, 1939, 33:213.

of salmonella strains in the United States will be found in papers by Seligmann, Saphra and Wassermann (1943), Rubenstein, Feemster and Smith (1944), Galton and Hardy (1918), and Felsenfeld and Young (1949).

In Denmark, during the years 1936-40, the types most often responsible for outbreaks of food poisoning were *Salm. typhi-murium*, *Salm. enteritidis* and *Salm. dublin* (Harhoff 1941). In all three countries, therefore, *Salm. typhi-murium* heads the list, and in Great Britain is responsible for three-quarters of all infections.

TABLE 138

TYPES OF SALMONELLA ISOLATED FROM CASES OR OUTBREAKS OF FOOD POISONING IN ENGLAND AND WALES. (From records of Salmonella Reference Laboratory.)

	1923-40	1941-5	1946	1947	1948	1949	1950	1951	1952
<i>Salm. typhi-murium</i>	279	712	573	473	663	1,053	1,593	1,236	1,604
<i>Salm. enteritidis</i>	72	135	28	35	43	51	91	99	145
<i>Salm. thompson</i>	74	87	38	28	57	82	67	97	90
<i>Salm. newport</i>	42	134	13	26	34	53	77	71	40
<i>Salm. cholerae-suis</i>	15	8	2	1	5	2	5	7	8
<i>Salm. bovis-morbificans</i>	11	18	2	9	14	13	23	14	6
<i>Salm. dublin</i>	13	9	9	22	22	24	40	23	17
<i>Salm. potsdam</i>	5	2	4	1	5	1	10	3	4
<i>Salm. senftenberg</i>	5	6	2	8	4	1	3	6	2
<i>Salm. derby</i>	6	7	1	—	—	1	7	5	6
<i>Salm. eastbourne</i>	2	—	—	1	—	—	—	—	—
<i>Salm. stanley</i>	3	1	—	—	4	8	1	4	19
<i>Salm. london</i>	10	—	—	—	1	—	—	3	4
<i>Salm. aberdeen</i>	5	1	2	—	1	3	—	1	—
<i>Salm. anatum</i>	—	20	4	4	9	8	20	11	10
<i>Salm. reading</i>	—	3	—	—	—	—	3	2	2
<i>Salm. oranienburg</i>	—	90	8	19	8	9	11	6	10
<i>Salm. montevideo</i>	—	62	16	13	17	9	18	23	12
<i>Salm. melleagridis</i>	—	24	2	1	1	2	3	—	2
<i>Salm. tennessee</i>	—	10	—	6	8	2	13	16	5
<i>Salm. bareilly</i>	—	6	1	7	2	1	14	5	15
<i>Salm. chester</i>	—	10	—	1	—	2	1	3	1
Other named types	2	40	15	10	10	31	45	67	79
Unidentified	23	61	28	24	—	37	44	9	61
Total	567	1,446	748	689	908	1,393	2,089	1,711	2,142

Note.—The figures refer to the number of cases or strains studied being due mainly to the inclusion of the double line were introduced during spray-dried egg.

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Besides members of the *Salmonella* group, certain organisms of the dysentery group, particularly *Sh. sonnei* and *Sh. flexneri*, may be concerned in the production of food poisoning. More usually these organisms give rise to dysentery, but occasionally their ingestion in food is followed by typical gastro-enteritis (Clayton and Hunter 1928, Sowden 1933, Scott 1936).

Animal Reservoirs of Salmonella.—Though human infection with *Salmonella* is widespread and frequent, the majority of food-poisoning outbreaks due to this group of organisms follow the consumption of food directly or indirectly associated

Egypt and Algiers. The quarantine stations at El Tor and Basra (see map) serve to keep the disease out of Europe in normal times though it seeps through into Iran and Iraq with some frequency. There was cholera in Europe during the Balkan War and the First World War and the disease occurred in epidemic form in Eastern Europe in 1921 and 1922. There has been no cholera in the United States proper since 1911.

As in the other enteric infections, the connecting link in the dissemination of cholera is between infected feces and the mouths of susceptible persons. In

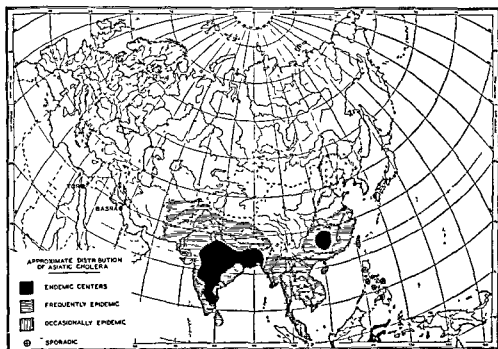


Fig 88. The approximate distribution of Asiatic cholera. The precise distribution cannot be shown because of great variability from time to time. This represents an approximate distribution during the decade 1930-1940. Based in part on map prepared by Army Medical Intelligence, 1943, epidemiological reports of the League of Nations, and various authors. (Based on Goode Base Map No 205. By permission of the University of Chicago Press.)

consequence, the disease is frequently water-borne and may be transmitted by any food ordinarily consumed in the raw state. The quantitative importance of contact infection is not known. Cholera differs from the other enteric diseases, however, in the highly explosive character of the outbreaks, which is attributable to the short incubation period, the high case fatality rate, and its rapid and permanent disappearance when the outbreak has subsided. The last is due in part, perhaps, to the inability of the vibrio to survive long apart from the host, but more important is the limited duration of the convalescent carrier state and the absence of chronic carriers. In a sense cholera is one of the easiest to control of the highly contagious diseases for it cannot spread when sanitary facilities, i.e., sewage disposal, water supply, etc., are in efficient operation. A striking instance of this was reported in the Balkan War in 1913 in which infection was widespread in the Bulgarian Army about Sofia, but cases in the

From these findings it may be concluded that birds, particularly chickens, turkeys, and ducks, are often infected with *Salmonella*. They constitute, in fact, the biggest known reservoir of salmonellæ. Practically every type so far met with has been isolated in cases of food poisoning in man.

Eggs may be infected during their formation in the oviduct, as in ducks, or by passage of the organisms through the shell from the feces on the exterior, as in hens and ducks (Scott 1933, Kathe and Lerche 1936, Lerche 1936, Jansen 1937). Penetration of the shell depends on a number of factors. If the shell is dry, little penetration will occur, and the bactericidal effect of the egg albumen will probably prevent growth of the few organisms that may pass through. When, however, a warm newly laid egg is brought into contact with fluid containing bacteria at a lower temperature than the egg itself, bacteria are readily sucked through the shell as the egg cools (Haines and Moran 1940). Whether multiplication occurs in the yolk is doubtful; but it is well to remember that, according to Prescott and Tanner (1938), most foods must be stored at 5° C. or below to ensure that no growth of these organisms takes place.

Pigs rival fowls in the frequency with which they are infected with *Salmonella*.

In Uruguay, Hormaeche and Salsamendi (1936) examined the pooled mesenteric lymph nodes from 20 normal animals at a time. *Salmonella* organisms were isolated from 22 out of 52 batches. An even higher proportion of positive results was obtained by the same authors (1939) in a later investigation. In this country, Scott (1940) examined pooled mesenteric lymph nodes and pooled spleens from apparently normal pigs, and isolated *Salmonella* from 36 out of 100 batches, representing an aggregate of 1,000 pigs. The lymph nodes were far more often infected than the spleen. A similar investigation undertaken during the war led to the isolation of 133 strains of *Salmonella* from 1,058 batches of lymph nodes representing a total of 5,285 pigs (Report 1947). Infection with a much wider variety of types was found in this investigation than in that by Scott, apparently as the result of feeding condemned batches of imported spray-dried egg to pigs. Smith and Buxton (1951) isolated salmonellæ from the feces of 4 out of 600 apparently healthy animals. In the Netherlands salmonellæ were cultured from 14/503 (2.8 per cent) of mesenteric lymph nodes (Clarenburg *et al.* 1949). In the U.S.A. Rubin, Scherago and Weaver (1942) found that about 10 per cent. of hogs in Lexington contained *Salmonella* in the mesenteric lymph nodes; and Cherry, Scherago and Weaver (1943) isolated salmonellæ from 5.9 per cent. of retail pig products, chiefly brain, chops and liver. In Mexico, Varela and Zoraya (1942) (see also Varela and Olarte 1952) isolated 15 strains of *Salmonella* from mesenteric lymph nodes taken from a total of 209 apparently healthy pigs. The types found in these different countries comprised *cholerae-suis*, *typhi-murium*, *anatum*, *thompson*, *newport*, *monterideo*, *oranienburg*, *oregon*, *derby*, *enteritidis*, *tennessee*, *senftenberg*, *gave*, *newington*, *barcilly*, *lexington*, *bredeney*, *new-brunswick*, *wothington*, *panama*, *dublin*, *bovis-morbificans*, *london*, *reading*, *norwich*, *chester*, *muenchen*, *heidelberg*, *meleagridis*, and *infantis*. In addition, Edwards and Bruner (1943) and Bruner and Moran (1949) include occasional strains of *paratyphi B*, *saint-paul*, *san-juan*, *kentucky*, *mississippi*, *poona* and *cerro*. *Salm. cholerae-suis* is probably the commonest type, because of the frequency with which it is found as a secondary invader in swine fever.

Cattle constitute a less important reservoir for *Salmonella* than either fowls or pigs; and the number of different types that have been isolated from them is considerably less.

Knoth (1936), Bartel (1938), Pohl (1938), and Lutje (1939) showed that the two commonest types in Germany are *Salm. dublin* and *Salm. typhi-murium*, but that other types, met with much less frequently, include *cholerae-suis*, *enteritidis*, *rostock*, *abortus-equi*,

of living vibrios; if peritoneal fluid is withdrawn from time to time and examined, it will be found that the vibrios lose their motility, become swollen and misshapen and then disintegrate. The phenomenon may be produced *in vitro* with potent antiserum and complement.

PARACHOLERA VIBRIOS AND NON-CHOLERA VIBRIOS

For the most part as an adjunct to the study of the cholera vibrio, a considerable number of vibrios have been isolated from water and from the feces of individuals suffering from mild diarrheal disease, so-called cholera nostra. Some of these have been given place names such as *Vibrio danubicus*, *Vibrio ghinda*, *Vibrio massachusetts*, etc. A phosphorescent vibrio, *Vibrio phosphorescens*, has been isolated from water, and *Vibrio proteus* from human feces. All of these differ immunologically from the cholera vibrio, i.e., belong to O Groups other than 1, and have faded from general interest. Some may possibly have feeble pathogenic properties but in tropical and subtropical regions where diarrheal disease is common (and of varied etiology) and great numbers of vibrios are present in water used for drinking, it is not surprising that they are occasionally found in fecal specimens though unrelated to a disease condition. In general, the group of vibrios is very poorly known from the biological point of view.

The El Tor Vibrios. The best known of the so-called paracholera vibrios are those which were first isolated at the Tor quarantine station in 1906 from pilgrims suffering from diarrheal disease. A considerable number of strains was isolated at Tor in 1930 and 1931 by Doorenbos, and apparently these vibrios can be isolated with some frequency there. Very many, though not all, of the El Tor strains are "agglutinable," that is to say, agglutinate with anticholera sera, and have been shown to belong to O Group 1 by Gardner and Venkatraman.⁴² These, like the cholera vibrio, contain both group- and type-specific O antigens, giving rise to El Tor vibrios of the Inaba type or Type AC, etc. The agglutinable strains differ from the cholera vibrio only in that they are actively hemolytic as noted earlier; a number of physiological tests, such as the Voges-Proskauer reaction, have been said to differentiate the two but none has proved to be reliable.

The relationship of these vibrios to Asiatic cholera has been a matter of considerable interest; aside from general considerations, their presence raises a specific practical question at quarantine stations. Doorenbos⁴² is strongly of the opinion that two types of cholera may be distinguished, the epidemic form caused by the classic cholera vibrio, and the endemic form caused by atypical variants of *V. cholerae* such as the El Tor vibrio; inherent in this is the assumption that the El Tor vibrios may become typical *V. cholerae*. This view is not generally accepted and it is believed that the El Tor vibrio, sometimes given the specific name *Vibrio El Tor*, has some pathogenic powers and may be causally associated with diarrheal disease, but neither the vibrio nor the disease is related to *V. cholerae* and Asiatic cholera in the sense of Doorenbos.

The Celebes Vibrio. The question of the pathogenicity of the hemolytic agglutinable vibrios was raised again by the occurrence of an epidemic, apparently of cholera, in Celebes in 1937 and 1938 which was reported by de

⁴² Doorenbos. Rev. d' Hyg. et de Med. Preventive, 1936, 58-595, 675, 736, *ibid*, 1937, 59:22, 105, Jour. Egypt. Med. Assn., 1938, 21:279.

by disturbances occurring at parturition, and 19-48 per cent. by other manifestations. It seems probable that meat from such animals is far more likely to be dangerous to human beings than meat from animals which are suffering from no more than a latent infection of the glands or spleen. This is in accordance with epidemiological evidence, which shows that in most instances of food poisoning due to the consumption of flesh from an infected animal, the animal has been "emergency-slaughtered." In Germany, such animals afford a frequent source of human infection. No fewer than 120 of the German outbreaks recorded by Meyer (1929) between the years 1923 and 1928 were due to this cause. In Great Britain and the United States, on the other hand, the consumption of meat from obviously sick animals is far less common, and most salmonella food-poisoning outbreaks are due to contamination of meat from a healthy animal after slaughter. Exceptions do, however, occur. Animals that appear to be healthy in the abattoir may nevertheless be suffering from a generalized salmonella infection. Scott (see Report 1936), for example, records an outbreak traced to the carcass of an animal that was almost certainly suffering from septicæmia. The carcass was not noticeably unhealthy, yet from all parts of it *Salm. typhi-murium* was isolated. Ketz (1937) likewise describes an outbreak due to the same organism which was traced to the meat of a young cow. This animal had been inspected by the veterinarian before slaughter and had been reported as in prime condition. No evidence of disease was found on examination of the carcass, but *Salm. typhi-murium* was isolated from a number of animals on the farm from which the cow came. A further exception must be made for certain milk-borne outbreaks in which either the udder of the cow is shown to be infected, or the milk becomes contaminated from the infected faeces of the animal. Outbreaks of the former type have been recorded by Kinloch, Smith and Taylor (1926) and Tulloch (1939), and of the latter type by McAllan and Howie (1931) and Conybeare and Thornton (1938). Healthy carriers among animals and birds are known to be common (see pp. 1801-04), but probably play little part in the genesis of food poisoning.

Passing on to the second method of infection, we may note that opportunities for sound meat to be contaminated during the course of preparation for food are numerous, though it is notoriously difficult to detect the way in which any given article has been infected. The main sources are rats and mice, and human carriers.

The frequency of rodent typhoid varies in different countries, and in different parts of the same country, and is often increased locally by the use of "virus" preparations containing living salmonella organisms for the extermination of rats and mice. It is well known that infected animals, while suffering from a chronic disease, or showing no obvious symptoms of illness, may excrete the causative organism in the faeces for weeks or months (Topley and Ayerton 1924). Moreover, in the faeces themselves, salmonellæ may survive under favourable conditions for 5 months or longer (Welch *et al.* 1941). Food prepared in unsanitary premises is liable to be contaminated from the droppings of these animals. It is usually difficult to obtain conclusive evidence that the food has been infected in this way, but outbreaks suggestive of a rodent source of infection have been described by Bainbridge (1912), Willführ and Wendtlandt (1921), Spray (1926) and Jones and Wright (1936). In addition, several outbreaks are on record in which infection has been traced, directly or indirectly, to the use of "virus" preparations (see Shibayama 1907, Jordan 1931, Kristensen and Bojlén 1931, Harhoff 1941, Leslie 1942, Dathan *et al.* 1947, Kokko 1947).

Moor.⁴³ About 400 strains of the vibrio were isolated and all proved to be hemolytic and agglutinable with cholera antiserum, van Loghem⁴⁴ found representative strains to be indistinguishable from the El Tor vibrio. With the outbreak of war in Europe and the Far East these strains were no longer available and do not seem to have been studied further. This epidemic, in which many cases were clinically indistinguishable from cholera though some were mild, is convincing evidence of a high degree of pathogenicity of at least some strains of the hemolytic agglutinable vibrios.

Other Pathogenic Vibrios. A number of other vibrios are known which produce disease in animals but which are apparently not pathogenic for man. *Vibrio metchnikovii* was isolated in 1888 from fowls suffering from an epidemic disease resembling fowl cholera. It closely resembles the cholera vibrio morphologically and physiologically and is highly pathogenic for guinea pigs and pigeons while the cholera vibrio is not pathogenic for the latter. It differs from *V. cholerae* immunologically and is neither agglutinated nor lysed by anticholera serum. An infectious abortion of sheep caused by *Vibrio fetus* was described by McFadyean and Stockman⁴⁵ in England and also occurs in this country but is not prevalent. The vibrio is relatively difficult to cultivate and is biochemically inactive, fermenting none of the sugars. It is not pathogenic for guinea pigs. An epidemic disease of carp and other fish caused by a vibrio designated *Vibrio piscium* has been described by David.⁴⁶ The vibrio resembled the cholera vibrio morphologically, and was immunologically related to it. A vibrio to which no formal name was assigned has been described by Doyle⁴⁷ as associated with swine dysentery. This vibrio was very difficult to cultivate and its relationship to the other vibrios is not as yet clear.

⁴³ de Moor. Bull. Office Internat. d'Hyg. Publique, 1938, 30:1510, see also de Vogel: *ibid.*, 1940, 32:556.

⁴⁴ van Loghem. Bull. Office Internat. d'Hyg. Publique, 1938, 30:1520.

⁴⁵ McFadyean and Stockman. Report of the Departmental Committee appointed by the Board of Agriculture and Fisheries to inquire into the disease of sheep known as "Abortus Ovis," 1909. Part I.

⁴⁷ Doyle. Amer. Jour. Vet. Res., 1944, 5:3.

How far the chronic human carrier is responsible for infection of food must, however, await further observation.

Before leaving this subject attention should be drawn to the apparent infrequency of salmonella food poisoning in man in spite of the widespread incidence of infection in domestic animals, and their products used for food, and in rodents. Scott (Report 1929) explained this discrepancy on the assumption that large doses of *Salmonella* were probably required for infection of man. In typhoid, cholera and probably dysentery, minute amounts of infective material may suffice to cause disease; but in food poisoning large doses are generally necessary. Savage (1942a) ably developed this theme in relation to paratyphoid fever; and Hormaeche, Peluffo and Aleppo (1936) supported it by human volunteer experiments with *Salm. typhi-murium*. These workers found that adults might ingest as many as 2,000 to 4,000 million organisms without suffering from any symptoms at all, or from not more than mild afebrile diarrhoea. Infants and old persons, however, appear to be considerably more susceptible, and fatal cases are restricted mainly to these two age groups.

Not too much attention should be paid to the doses just mentioned. The infecting dose probably varies greatly with the strain of organism, the medium in which it has been grown, and the susceptibility of the human subject. McCullough and Eisele (1951a, b), who carried out feeding experiments on volunteers in prisons, found a great variation in the dose required to produce illness. In some men illness was caused by as few as 125,000 organisms of *Salm. bareilly* and 152,000 of *Salm. newport*, though others resisted doses of 1,700,000 and 1,350,000 respectively. None of the volunteers that ingested *Salm. derby* became ill till a dose of 15,000,000 organisms was reached. One strain of *Salm. anatum* had an infecting dose of under 1 million, whereas another strain had an infecting dose of about 50 million. With *Salm. meleagridis* the infecting dose ranged from 7 to 24 million. Symptomless infection, as judged by faecal excretion, was often set up with much smaller doses than those required to cause illness.

Staphylococcal Toxin Poisoning

As Dack (1949) pointed out, the ability of some strains of staphylococci to produce a toxic substance capable of irritating the gastro-intestinal tract has been discovered four times—by Denys (1894) in Belgium, by Owen (1907) in the United States, by Barber (1914) in the Philippines, and by Dack, Cary, Woolpert and Wiggers (1930) in the United States. In 1914 Barber, working in the Philippines, reported a very clear instance of a "toxin" outbreak due to the growth of a white staphylococcus in the milk of a certain cow. Immediately after withdrawal from the udder, the milk could be consumed with impunity, but when consumed after standing for a few hours at room temperature, 28°-30° C., it gave rise within about 2 hours to nausea, vomiting, abdominal pain, diarrhoea, cramps, and faintness. Similar symptoms were produced in human volunteers by the consumption of pure milk cultures of the strain of *Staph. albus* isolated from the cow in question, a strain which was highly refractory to heat. This report furnished in itself a strong case for the causative factors responsible for a "toxin" outbreak. Unfortunately, however, no particular attention seems to have been paid to it, and it was not till the rediscovery several years later by Dack and his colleagues (1930) of staphylococcal food poisoning that the subject attracted the attention it deserved. Since that time

BRUCELLA¹

Undulant Fever, Contagious Abortion of Cattle

In 1887 Bruce, while investigating the human disease known as Malta fever, Mediterranean fever or undulant fever, discovered a microorganism in the spleen of fatal cases of the disease which he designated *Micrococcus melitensis*. A disease of goats transmissible to man, this affection not only is common on the island of Malta, where British garrisons have been often seriously affected, but occurs also on neighboring islands and on the shores of the Mediterranean Sea, and has been occasionally reported from India, South Africa, the Philippines and the West Indies. It was first brought to attention in the United States about 1911.

In 1897 Bang, in Denmark, isolated a microorganism responsible for a contagious abortion in cattle, an affection now commonly known as Bang's disease, which he termed *Bacillus abortus*. The isolation and cultivation of this bacterium in the United States were first recorded by MacNeal and Kerr in 1910.

These two diseases, one primarily of goats and secondarily of man, and the other, one of cattle, were long studied quite independently, and apparently no connection between the two was recognized prior to the work of Evans² in 1918. This worker demonstrated the remarkably close morphological, cultural and serological relationship existing between these bacteria which are now recognized as being intimately related to one another.

In 1914 Traum isolated from fetuses prematurely expelled from sows a bacterium which is now known to be closely related to the bacterium of Bang's disease and that of undulant fever. Regarded as three species, these bacteria have been given the generic name of *Brucella* and are designated as *Brucella melitensis*, *Brucella abortus* and *Brucella suis*. Infection with these bacteria is often termed "brucellosis."

Morphology and Staining. The *Brucella* are small coccoid or short bacillary forms varying from 0.4 to 3.0 μ in length and from 0.4 to 0.8 μ in breadth. Some variability is noted, and both coccoid and bacillary forms may appear intermingled. There is a greater tendency to the coccobacillary form in *Br. melitensis* than in *Br. suis*, with *Br. abortus* intermediate be-

¹ For a detailed discussion of these bacteria see Huddleson *Brucellosis in Man and Animals*. 2nd ed. The Commonwealth Fund, New York. 1939.

² See the general discussion by Evans. *Amer. Jour. Pub. Health*, 1947, 37:139.

The enterotoxin gives rise to acute gastro-enteritis with severe collapse when consumed by human volunteers in doses of 2-10 ml., but different persons vary in their susceptibility towards it. It is practically non-toxic when fed to most laboratory animals, though young *rhesus* monkeys may be affected after a dose of 25-50 ml. It is of doubtful antigenicity, and little tolerance to it seems to be developed in the human subject as the result of repeated dosage (see Jordan 1930, Jordan, Dack and Woolpert 1931, Dack, Jordan and Woolpert 1931a, Dack *et al.* 1931b, Jordan and Burrows 1933); though Dolman (1944) claims to have been able to immunize human subjects against it by repeated subcutaneous injections of a formol toxoid preparation.

Food Poisoning due to *Clostridium welchii*

How *Cl. welchii* acts is still not clear, but no doubt exists any longer of the rôle of this organism in causing one characteristic form of food poisoning. During the war several outbreaks occurred in children partaking of school dinners prepared in a communal kitchen (Knox and Macdonald 1943). Symptoms began after an incubation period ranging from 8 to 18 hours, and consisted of nausea, abdominal cramps and diarrhoea lasting for about 12 hours. Inquiry revealed that a meat dish had been prepared on the previous day, allowed to cool down overnight, and served up after warming. On examination in the laboratory the meat or gravy was found to contain enormous numbers of *Cl. welchii*. The cooking of the meat had destroyed all non-sporing organisms and had driven off the oxygen. The surviving spores of anaerobic bacilli, finding themselves in a medium containing glutathione and other reducing substances, had germinated as the temperature fell and then multiplied in practically pure culture. The resulting dish was undoubtedly poisonous, but whether owing to the organisms themselves, or to a specific toxin produced by them, or to some non-specific breakdown product of the meat could not be ascertained.

Since the war this form of food poisoning has been increasingly recognized; in 1950 no fewer than 24 outbreaks, confirmed by laboratory examination, were reported. It has also been met with in the United States (McClung 1945). According to Hobbs and her colleagues (1953), who made a special study of it, the responsible organism is a variant of Type A, differing from the modal organism in its non-hæmolytic colonial appearance, its feeble toxigenicity, and the heat resistance of its spores. It can be isolated from the faeces of about 90 per cent. of those affected, but is present in only about 2 per cent. of normal persons.

In Germany a disease known as *enteritis necroticans* was described (Zeissler *et al.* 1949a, b, Oakley 1949, Willich 1949). It has an acute onset, is characterized by abdominal pain, vomiting and diarrhoea, and may be rapidly fatal. *Post mortem* there is a diffuse sloughing enteritis of the jejunum, ileum and colon. A new type of *Cl. welchii*, referred to as Type F, was isolated from the food and from the patients' faeces; the spores were heat-resistant and withstood boiling for 1-4 hours (See also Chapter 78).

Non-Specific Bacterial Food Poisoning

Numerous outbreaks have been described in which no organisms of the *Salmonella*, *Shigella*, *Staphylococcus* or *Clostridium* group could be isolated, but in which the incriminated food was found to contain large numbers of organisms belonging

tween the two, but no distinction can be made on a morphological basis. The microorganisms usually occur singly or in pairs, and in cultures short chains may be found. The smooth forms are encapsulated but spores are not formed, and these bacteria are non-motile.

On semisolid media the colonies are small, circular, convex, amorphous, smooth, glistening and translucent. No pigment is formed, but the growth of *Br. melitensis* becomes brown in older cultures and the browning extends down into the medium. This browning is shown by some strains of *Br. abortus* also.

Brucella may be stained by the usual aniline dyes, but there is a tendency toward irregular staining and, in some cases, bipolar staining. They are gram-negative.

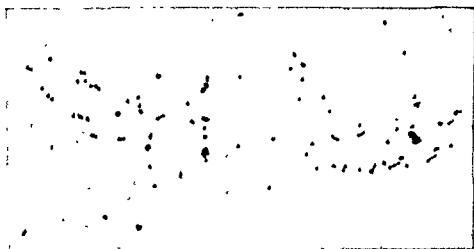


Fig 90. *Brucella melitensis*, pure culture. Note the coccobacillary appearance Fuchsin, $\times 1050$.

Physiology. The nutritive requirements of these bacteria are relatively complex and best growth is obtained on enriched media such as liver infusion broth or agar. Kerby and Calder³ have reported that a milk-tryptose-crystal violet medium is superior to liver infusion broth or tryptose broth in primary culture. *Brucella* have been cultivated on amino acid synthetic media, some strains require nicotinamide, thiamine and pantothenic acid while others require biotin also. A number of chemically defined or synthetic media have been prepared which support the growth of *Brucella*, one of the simplest of which is that of Gerhart and Wilson⁴ containing lactate, glycerol, asparagin (or glutamic acid or histidine), thiamine, nicotinic acid, pantothenic acid and biotin together with inorganic salts. No growth occurs at 6° C. or 45° C. and the optimum temperature is 37° C. Neither acid nor gas is produced from carbohydrate media, although it may be shown that glucose is utilized to a small extent and its inclusion generally favors growth. Nitrates are re-

³ Kerby and Calder. Jour. Bact., 1940, 40 637.

⁴ Gerhardt and Wilson. Jour. Bact., 1948, 56 17, see also the studies of McCullough et al.: *ibid.*, 1947, 53-5.

staphylococcal hæmolyisin in an extract of food. Moreover the incubation period in many of these outbreaks is too long to be characteristic of staphylococcal enterotoxin.

Whether organisms of the *Salmonella* group can produce enterotoxic substances remains open to dispute. There is plenty of evidence to show that they contain in their bodies thermostable, water-soluble substances which are toxic on parenteral inoculation into animals (Brieger and Fraenkel 1890, Cathcart 1906a, Ecker 1917, Savage and White 1925a, Menten 1926, Bahr and Dyssegaard 1927, Meyn 1930). Methods for extracting these bodies have been described by Boivin and Mesrobian, Raistrick and Topley, and Morgan (see Chapter 44), who have shown that they consist of lipopolysaccharide. Injected intravenously into rabbits, they give rise to great weakness and prostration, often accompanied by tremors, diarrhœa, and hyperglycæmia (Delafield 1934); and injected intraperitoneally into mice in a dose of about 0.5 mgm., they prove fatal (Martin 1934). But there is as yet no conclusive evidence that they are able to cause gastro-enteritis in man when taken by the mouth. Feeding experiments carried out on human volunteers and on monkeys by Dack, Harmon and Jarra (1928), Dack, Cary and Harmon (1928), and Verder and Sutton (1933), using heat-killed organisms or culture filtrates of *Salm. typhi-murium* and *Salm. enteritidis*, proved uniformly negative. Moreover, the ingestion by students of even 200 mgm. of the lipopolysaccharide fraction isolated by Raistrick and Topley (1934) from *Salm. typhi-murium* was without any obvious effect (Colbeck 1942).

Whatever the cause of the non-specific type of bacterial food poisoning, there is no doubt that in Great Britain it is a common type. The figures for 1950 show that it constituted almost half of the reported outbreaks. The real proportion is probably much higher, because an attack of the disease, though sometimes severe, is usually soon over and the doctor may not be called in. Moreover, since in many cases and outbreaks the bacteriological findings are completely negative, no further attention is paid to them.

Diagnosis and Investigation of Outbreaks.

The diagnosis of food poisoning is primarily clinical. For the full investigation of an outbreak the reader is referred to textbooks of hygiene, but the general procedure is briefly as follows: (1) Secure a complete list of cases. (2) Obtain particulars of individual cases. (3) Ascertain the vehicle of spread. (4) Determine the causal agent—chemical irritant, *Salmonella*, *Shigella*, *Cl. botulinum*, staphylococcal toxin, or other form of toxin or infection. (5) Find out how the vehicle became infected. (6) Endeavour to trace the reservoir from which the vehicle became infected, such as rodents, cattle, fowls, ducks, or human carriers (see Savage 1942b).

Materials collected for examination should include (1) the actual food consumed; it is most important that this should be obtained: (2) the vomit and fæces of patients: (3) the blood, spleen, liver and intestine of fatal cases: (4) the fæces of suspected carriers who may have contaminated the food. The material should be sent to the laboratory packed in ice. A bacteriological examination should first be carried out, and if this is negative, search should be made for chemical poisons or preservatives in the food.

In salmonella outbreaks the organisms can frequently be demonstrated in the food, and in the fæces of the patient; vomited matter is much less satisfactory. In their isolation the general plan should be to plate suspensions of the suspected

duced, and growth in milk is accompanied only by a slowly increasing alkalinity. Gelatin is not liquefied and indol is not formed. The optimum pH is 6.6 to 6.8.

Hydrogen Sulfide. All three species produce hydrogen sulfide but differ in that *Br. suis* produces it in abundance, *Br. abortus* to a lesser extent and *Br. melitensis* to only a slight degree. It may be noted that ammonia is produced to a greater extent by *Br. melitensis* than by the other two species.

Carbon Dioxide. These bacteria are aerobic, and *Br. melitensis* and *Br. suis* may be grown on primary isolation under the usual aerobic conditions. *Br. abortus*, however, requires incubation in an atmosphere containing 10 per cent carbon dioxide on primary isolation. Subsequent transfers from the primary growth must be incubated in 10 per cent carbon dioxide, but after a number of transfers *Br. abortus* adapts itself to ordinary aerobic growth.

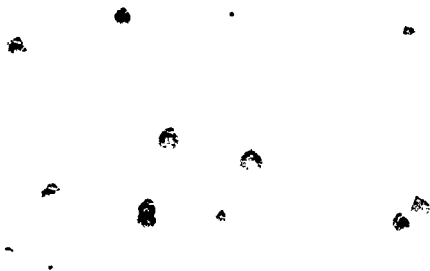


Fig. 91. *Brucella melitensis*. Pure culture on liver infusion agar. $\times 4$.

Dyes. The *Brucella* species differ from one another in their susceptibility to the bacteriostatic effect of dyes, and their ability to grow on liver infusion agar containing thionine or basic fuchsin is a characteristic of considerable practical value in their differentiation. *Br. melitensis* and *Br. abortus* will grow in the presence of basic fuchsin in a dilution of 1:25,000, but *Br. suis* is completely inhibited. *Br. melitensis* and *Br. suis* will grow in the presence of thionine in a dilution of 1:50,000, while *Br. abortus* will not. These and other cultural differences are summarized in the accompanying table.

The *Brucella* show the usual susceptibility to heat and disinfectants. A point of some practical importance is the rapid death of these bacteria at pasteurizing temperatures, both *Br. abortus* and *Br. suis* are killed in three minutes at 143° to 145° F. They persist in soil, water and dust for one to two months but disappear within ten days in milk, presumably in part as a consequence of the presence of acids formed by other bacteria. In this connection it is of interest that these bacteria are able to survive two hours or more in milk mixed with gastric juice.

pointing to chemical poisoning, an endeavour should be made, particularly in outbreaks of the toxin type, to obtain evidence of the presence of toxic substances of bacterial origin in the food or of organisms capable of forming such substances. The investigation of this type of outbreak is remarkably difficult. Though it may be worth while feeding the suspected material to *rhesus* monkeys, the only satisfactory way is to make tests on human volunteers. Search should be made in the food for organisms such as staphylococci, streptococci, coliform, *Proteus* and aerobic spore-bearing bacilli, which may be potentially toxigenic; but little attention should as a rule be paid to any of these organisms unless it is present in large numbers. Similar organisms should be sought for in the patient's excreta. In staphylococcal outbreaks, the organisms may be found in the vomit and occasionally in the faeces; if so, they should be examined serologically (Hobbs 1948) or by the bacteriophage typing method (Wilson and Atkinson 1945) to see whether they resemble the strain isolated from the food. Several outbreaks are on record in which the probable identity of the strains isolated from the food, the patients' vomit or faeces, and the food handler has been demonstrated by phage-typing (see Wilson and Atkinson 1945, Saint-Martin *et al.* 1951, Williams *et al.* 1953). Any organism on which serious suspicion is cast should be grown in soft agar for 2-3 days in 10-30 per cent CO₂, and a saline extract of the culture, filtered or centrifuged to remove living organisms, fed in 2-5 ml. quantities to human volunteers.

It should be pointed out that, in staphylococcal poisoning due to cooked food, very few staphylococci may be demonstrable. This was true, for example, of a series of outbreaks traced to spray-dried skim milk. Though most of the organisms had been destroyed by the processing, sufficient of the heat-stable enterotoxin was present to cause poisoning when the milk was used almost immediately after reconstitution.

In many of these outbreaks even a careful review of all the evidence, epidemiological and bacteriological, will justify no more than a cautious expression of opinion as to their mode of origin. As has already been pointed out, there is ample scope for investigation of this type of outbreak, and it is to be hoped that in the future an attempt will be made to ascertain the chemical nature of the bacterial toxins found in the food.

The examination of canned foods should be conducted on the same principles as those of fresh food, but there are a number of technical details to be attended to; for a description of these the reader is referred to articles by Esty and Stevenson (1925), Savage (1923), Esty (1935), Woodcock and Lewis (1938), Jones and Jones (1941), Report (1941), and Baumgartner (1943). Methods for the bacteriological examination of brined, salted and pickled vegetables are described by Etchells and Jones (1946).

Prophylaxis.

The hygienic precautions necessary to prevent food poisoning concern the whole course of the food from the slaughter of the animal to the final preparation for consumption. A thorough system of meat inspection is essential. The meat of animals that are ill or are emergency-slaughtered should as a rule be condemned. To this precaution alone Meyer (1916) attributes the comparative infrequency of meat poisoning in California, where it is known that calves are infected with *Salmonella enteritidis*. The laxity of the meat inspection in Germany since the war of 1914-18 appears to have resulted in a great increase in the number of poisoning outbreaks. The measures to be taken to ensure the cleanliness of premises where made-up

Antigenic Structure. Each of the three *Brucella* species contains two antigens, designated as A and M. *Br. melitensis* contains a relatively large amount of M and a small amount of A, while both *Br. abortus* and *Br. suis* contain large amounts of A and small amounts of M.⁵ The ratio of A to M is said to be about 20:1 in the case of *Br. abortus* and 1:20 in the case of *Br. melitensis*. It is possible, then, to differentiate *Br. melitensis* from *Br. abortus* and *Br. suis* by serological methods, i.e., agglutination, but *Br. abortus* and *Br. suis* cannot be differentiated from one another in this way. In practice monospecific sera, i.e., sera adsorbed to remove the small amount of antibody common to the other immunological type, should be used. These antigens are both heat-stable, and their chemical nature is as yet unknown.⁶

CULTURAL DIFFERENCES OF BRUCELLA SPECIES

	CO ₂ Required for Growth	H ₂ S Production	Growth in Presence Of	
			Basic Fuchsin 1 25,000	Thionine 1 50,000
<i>Br. melitensis</i> .	—	±	+	+
<i>Br. abortus</i> .	+	+	+	—
<i>Br. suis</i> .	—	+++	—	+

Variation. The *Brucella* species dissociate relatively easily to give rise to the rough form. The environmental factors affecting the dissociative process have been studied in detail by Braun.⁷ The S-R transformation is accompanied by a change to a rough colonial type, a loss of virulence and alteration in immunological specificity. The last begins to take place before morphological changes are apparent and has been a source of considerable difficulty in the serological typing of these bacteria; the altered immunological types have been termed *paramelitensis* or *para-abortus* strains. It is, therefore, essential to use only smooth cultures in the serological differentiation of these bacteria.

A number of tests have been devised for the detection of antigenic variants. The rough forms are, of course, spontaneously agglutinated in saline. Slightly rough strains may be detected by their agglutination upon boiling in saline for two hours (thermo-agglutination test) or by incubating saline suspensions of the bacteria with basic fuchsin or trypanflavine at 37° C. for two hours, the rough form agglutinates in the presence of the dye while the smooth form remains evenly suspended. According to Huddleson,⁸ however, none of these gives consistent results, and he has proposed an

⁵ Wilson and Miles. Brit. Jour. Exp. Path., 1932, 13 1.

⁶ Cf. the investigations of Miles and Pirie Brit Jour Exp Path., 1939, 20 83, 109, 278, Biochem. Jour., 1939, 33 1709, 1716.

⁷ Braun. Jour. Bact., 1946, 51 327.

⁸ Cf. Munger and Huddleson. Jour. Bact., 1938, 35 255.

Duck eggs should be boiled for 15 minutes (Clarenburg and Burger 1950); frying and other procedures that do not ensure coagulation of the yolk cannot be relied upon to destroy all salmonellæ. Spray-dried egg often contains salmonellæ (Report 1947); it should not be reconstituted till just before use and should be thoroughly cooked. Laboratory experiments show that the mixture of yolk and white of egg from which it is prepared can be satisfactorily pasteurized (Winter *et al.* 1946); it is hoped that, in time, this method may be generally adopted.

The protection of custard and cream fillings and of made-up meat dishes, such as brawn and pressed beef, from staphylococcal infection of human origin requires the greatest care and vigilance. In heavy nasal carriers of *Staph. aureus*, the hands are often infected, and there may be suppurative skin lesions on the hands and arms. Measures little short of a surgical aseptic technique may have to be taken to prevent infection of the ingredients, and stringent regulations should be laid down about the adequate heating and subsequent refrigeration of the food. For preventing the growth of salmonellæ, staphylococci and other pathogenic organisms in synthetic cream, the addition of 0.005-0.02 per cent. of hydrogen peroxide has been recommended (Hobbs and Smith 1951.)

BOTULISM

INTRODUCTORY

Botulism (from Latin *botulus*, a sausage), sometimes known as allantiasis, or ichthyosismus, is a disease that was first described in 1820 by the German poet and medical writer Justinus Kerner (Jordan 1917). The causative bacterium was isolated and described by van Ermengem in 1896 (1896, 1897). After a festive gathering of a music club at the village of Ellezelles in Belgium, several of the members were taken ill, and three of them died within a week. The disease was confined to those who had partaken of a certain piece of raw ham; the other ham and the remainder of the animal had been previously consumed without causing trouble. The contaminated ham was paler and softer than normal and smelt rancid. When fed to cats it caused mydriasis, partial paresis, secretory disturbances, aphonia, and other symptoms. From it van Ermengem cultivated a strictly anaerobic organism, which secreted a powerful toxin, giving rise to the same symptoms in cats as those caused by the original ham.

Since that date several outbreaks of botulism have been recorded, some in Germany, but more in America. In this country only four small incidents have been described (see p. 1818).

Symptomatology and Pathology.

The incubation period is generally under 24 hours after consumption of the affected food, but may be prolonged to 72 hours. Prominent among the symptoms are vomiting, constipation, ocular pareses, thirst, pharyngeal paralysis, the secretion of thick, viscid saliva, and sometimes aphonia. General consciousness and sensibility remain intact till near the end, which is preceded by coma or delirium. The temperature is generally subnormal—96-98° F. Later in the illness it may rise owing to the onset of broncho-pneumonia (Dickson 1918). Death may occur within 24 hours from the time of onset, or may be delayed for a week. In cases that survive, complete recovery, particularly of the ocular movements, may not

opsonocytophagic test in which bacterial suspension is mixed with citrated normal guinea pig or human blood, incubated for thirty minutes, smeared and stained. Smooth strains show only a slight degree of phagocytosis while the rough bacteria are phagocytosed to a considerable extent.

Pathogenicity for Lower Animals. Brucellosis is primarily a disease of domestic animals and is only secondarily communicated to man, the chief animal reservoirs are goats, cattle and swine. It is of some interest that host specialization of the parasites has taken place, giving rise to the three species or, as some prefer to regard them, varieties of *Brucella*.

Goats. Goats may be artificially infected with *Br. melitensis* by almost any route, and it is probable that under natural conditions the vaginal discharge at the time of aborting and shortly thereafter plays an important part in the dissemination of the infection. Agglutinins appear in the blood of artificially inoculated pregnant goats by the third or fourth day, and the titer rises rapidly to perhaps 1:1000 within forty-eight hours and reaches a peak of 1:2000 or thereabouts by the twelfth day of infection. Just before the peak in agglutinin titer, a bacteremia is initiated which persists for perhaps one month. This acute generalized infection becomes localized during the second month after the termination of the pregnancy during which the animal was infected. In most cases the bacteria do not persist in the udder and uterus after the fifth month following termination of pregnancy. A second pregnancy does not, as a rule, cause an exacerbation of the disease, but in some cases the infection may remain localized in the area of the genital tract for several years.

The most obvious clinical symptom of infection is abortion, although this need not occur. Pyrexia is apparent within forty-eight hours of the generalized infection, and there is a slight diarrhea. The placenta is not retained, but a copious vaginal discharge is frequently observed for two or three weeks after kidding. In lactating goats the milk may be physically altered and appear in extreme cases as a clear fluid containing suspended clots.

Immature goats are highly resistant to the infection, and kids born of infected dams may not be infected and commonly do not become so in spite of the ingestion of enormous numbers of *Brucella* in the milk. Non-pregnant mature goats are also resistant to infection and respond to artificial inoculation with only a low and transient agglutinin titer in the blood serum.

Brucellosis in sheep is similar to that in goats.

Cattle. Brucellosis in cattle is most commonly an infection with *Br. abortus* although both *Br. melitensis* and *Br. suis* have also been found. The microorganism may gain entrance by a variety of routes, including direct inoculation into the vagina, by way of the conjunctiva, through the unbroken skin or via the alimentary tract. The primary symptom of the disease is abortion of the fetus by pregnant cattle.⁹ The time elapsing between initial infection and abortion varies from three weeks to four months, and the period of gestation at which abortion may take place varies from

⁹ Abortion may, of course, result from other infections. See the discussion by Gilman: *Cornell Vet.*, 1939, 29:153.

1923, Starin and Dack 1925) that if spores, which have been freed of their toxin by repeated washing or by heating to 80° C. for half an hour, are injected subcutaneously into guinea-pigs in large doses—500 million or so—or are given by the mouth in still larger doses—about 2,000 million—they may give rise to fatal disease; from the spleen, liver, kidneys, or mesenteric glands the bacilli may be recovered in pure culture after death. The experiments on which these findings are based are not very convincing; doubtless after such massive doses the spores are disseminated throughout the body. But it is doubtful whether germination of the spores occurs or whether the resultant bacilli multiply and form toxin. Since it is extremely difficult to free spores completely from all traces of toxin, especially by washing, and since very small quantities are required to kill a guinea-pig—1/10,000 mgm. or less—it is not surprising that some animals injected with so-called toxin-free spores succumb to the disease.

Even if it is possible experimentally to reproduce a true infection with *Cl. botulinum*, there is no evidence that under natural conditions the disease in man is other than a pure intoxication. In this view the majority of authors strongly concur (van Ermengem 1897, Römer 1900, Armstrong *et al.* 1919, Burke *et al.* 1921, Geiger *et al.* 1922).

A disease closely simulating botulism in man can be reproduced by the experimental inoculation or feeding of cats, monkeys, and certain other animals with toxic material (see Chapter 36).

Epidemiology.

Botulism is due to the consumption of food in which *Cl. botulinum* has been growing. It occurs therefore in sporadic outbreaks limited to those who have partaken of the contaminated food. Since the disease is an intoxication, not an infection, secondary cases do not occur, but it is common for cats, dogs, and especially chickens that are fed on the remnants of the food to develop symptoms of poisoning.

Botulism is not a common disease. From 1899 to 1925, 146 outbreaks were reported in the U.S.A. and Canada, affecting 504 persons; of these 337 died—a case-fatality rate of 67 per cent. (Editorial 1926). Bacteriological evidence of the nature of the disease was available in 53 of these outbreaks. These figures probably underestimate the incidence of the disease. During the 8 years 1918–25 in the U.S.A. there were about 13 outbreaks per annum.

In Germany 24 outbreaks of suspected botulism were reported between 1907 and 1923, but in only one of these was *Cl. botulinum* isolated. There is said to have been an increase in the frequency of the disease since the second world war, partly attributable to the greater consumption of pickled fish (Wasmuth 1948). In the British Isles only 4 incidents have been recorded, the largest of which occurred in 1922 at Loch Maree in Scotland, when 8 persons were attacked after eating potted duck paste, all the patients died within a week. *Cl. botulinum* Type A was cultivated from the remnants of the paste (Leighton 1923). The remaining three were in London. Two (Templeton 1935, Lane and Jones-Davies 1935), of which one was shown to be due to Type A, followed the consumption of vegetarian nut brawn. The third (Aitken, Barling and Miles 1936), which was due to Type B, occurred after eating a home-made meat pie. In America the disease is commoner during the winter months—the season of preserved food consumption.

Morbidity and Fatality Rate.—In any outbreak the morbidity rate is high. As a rule all who partake of the contaminated food develop the disease. The case-fatality rate varies in different outbreaks. In Germany it is said to be not more

two to nine months. Cattle do not abort, however, unless infected during pregnancy and even then not all abort—perhaps 30 per cent—or the cattle may become sterile. Subsequent pregnancies may proceed normally in spite of persistence of the infection, second abortions are not common, and third abortions are rare.

The bacilli may be found in the blood in perhaps 10 per cent of the cases and are very likely consistently present during the acute infection. Early in the infection the bacteria are found in the lymph glands about the head and intestines, by the end of the first month they are found all through the body, and by the end of the third month have localized in the mammary glands and are found only in the udder. The invasion of the udder results in an acute or chronic inflammation with lesions in the alveoli and interalveolar connective tissue and, when the lymph glands are involved, a chronic lymphadenitis. Chronic infection of the udder may persist indefinitely without significant differences in the quality of the milk and bacilli may be excreted over a long period of time, perhaps for life. The uterus, on the other hand, frees itself of the bacteria relatively soon and the vaginal discharges do not contain the bacilli for an extended period.

Animals infected during pregnancy show an agglutinin titer ranging from 1:200 to 1:1000 which falls slowly over a period of six months or so. Cattle that continue to excrete bacilli in the milk generally show persistent agglutinin titers of 1:200 or more although a titer of 1:50 has diagnostic significance. Agglutinins are also present in the milk and may be demonstrated in the whey after clotting with rennin. Infected animals become sensitized to the bacillary substance, and a skin reaction may be elicited by the intradermal injection of a preparation of *Brucella* protein designated as *abortin* or *brucellergen*. As in the case of the young goats, calves are relatively resistant to the infection.

Swine.¹⁰ Brucellosis of swine seems to be always due to infection with *Br. suis*, though these animals may be artificially infected with *Br. abortus*. In contrast with cattle, the males are commonly infected and abortion in infected females is less frequent than in cows; about 50 per cent of swine abortions are due to unknown causes, not brucellosis. The clinical symptoms may be mild or lacking, and in a number of instances there has been no outward evidence of the disease in an infected herd but the proportion of swine infected is as high as 20 per cent in some localities. Under natural conditions infection probably takes place via the alimentary tract. The bacilli are eliminated with aborted fetuses and vaginal discharge, urine, semen and milk.

Other Animals. A number of other animals have been found to be naturally infected with *Brucella*. There is some evidence that the disease of horses known as fistula of the withers or poll evil is a *Brucella* infection; both *Br. abortus* and *Br. suis* have been isolated from cases of the disease. Stone¹¹ found that 9.5 per cent of horses tested in New York City gave positive serological reactions. *Brucella* infections of fowl have been reported, in one instance *Br. suis* was isolated from several naturally infected

¹⁰ See the general discussion by Hutchings, Mich. State Coll. Vet., 1944, 4:68, 69, 86.

¹¹ Stone, Jour. Amer. Vet. Med. Assn., 1941, 99:118.

the organism is a natural inhabitant of the soil and not merely a contaminant derived from animal faeces. Indeed the older view that the organism is a natural inhabitant of the intestine of animals, and is spread by their faeces, must be abandoned.

Meyer and Dubovsky (1922c) likewise examined specimens of soil from Belgium, Denmark, the Netherlands, England, and Switzerland. *Cl. botulinum* Type B was demonstrated in a variable proportion of soils from the different countries, but in not a single instance was Type A found. From this country 64 specimens were examined, and 9 of them yielded toxic cultures; the counties furnishing the positive results were Durham, Derbyshire, Middlesex, Hereford and Sussex, thus indicating a widespread distribution

TABLE 139

DISTRIBUTION OF *Cl. botulinum* IN DIFFERENT TYPES OF SOILS IN ALL STATES OF AMERICA, WITH THE EXCEPTION OF CALIFORNIA AND VIRGINIA.

Modified from Meyer and Dubovsky (1922b)

	Specimens Examined	Toxic Cultures.	Weak Toxin	Type A.	Type B.	Types A and B
Virgin soil	335	105	22	59	22	2
Cultivated soils	274	47	13	18	16	—
Garden soils	142	41	12	23	6	—
Soil and manure from animal corrals and yards .	161	20	9	6	5	—
Pasture	51	19	5	3	11	—

of the organism. In Scotland, Leighton and Buxton (1928), in an examination of 100 samples of soil, encountered *Cl. botulinum* four times; two of the cultures belonged to Type A, one to Type B, while the remaining one contained both Types A and B. Haines (1942), who examined 106 samples of soil from different parts of England—mainly the south-east—isolated *Cl. botulinum* with certainty from 5 per cent. Of the 5 strains, 4 came from grassland; 4 were Type A and 1 Type B. Both types of *Cl. botulinum* have been demonstrated in the soils of Canada, China, and at least one of the Pacific Islands (Dubovsky and Meyer 1922b, Schoenholz and Meyer 1922).

TABLE 140

DISTRIBUTION OF *Cl. botulinum* IN DIFFERENT VEGETABLES AND FODDER IN ALL STATES OF AMERICA, WITH THE EXCEPTION OF CALIFORNIA AND VIRGINIA.

Modified from Meyer and Dubovsky (1922b).

	Total No. Examined.	Positive Cultures	Percentage of Positive Cultures
Mouldy hay	44	7	15.9
String beans, pods and stalks	44	14	31.8
Decayed vegetation	29	6	20.6
Ensilage	15	3	20.0
Corn husks, leaves and stalks	80	6	7.5
Beets, roots and tops	37	6	16.2
Tomato plant and roots	24	2	8.3

Meyer and Dubovsky's results have not as yet received general confirmation; some of their conclusions may have to be modified (Geiger and Benson 1923, Bachmann and Haynes 1924), and more work must be carried out before the relationship of the two types to environmental conditions can be definitely determined.

birds, but the disease is probably not common. Dogs may also be infected naturally; *Br. suis* and *Br. abortus* have been isolated. Wild rats may be artificially infected, and *Br. abortus* has been isolated from a naturally infected rat. Natural infection of rabbits with *Br. melitensis* has also been reported.¹² Of the usual laboratory animals guinea pigs are readily infected and are most often used for experimental purposes. Rabbits, mice and other animals may also be infected. A disease resembling undulant fever in man has been produced in rhesus monkeys.

Pathogenicity for Man. Man is susceptible to infection with these three species of *Brucella*, but infections with *Br. melitensis* and *Br. suis* are usually more severe than those with *Br. abortus*. The incubation period of undulant fever in man is highly variable and relatively long; it may range from one week to not less than four months. The case fatality is low—2 to 3 per cent. It may have varied clinical manifestations and 5 types are recognized: (1) the intermittent type with shifting articular rheumatism, weakness, night sweats and a temperature near normal in the morning but rising to 101° to 104° F. in the evening, in which the patient remains in bed in the latter part of the day; (2) the ambulatory type with much the same symptoms but to a mild degree; (3) the undulant type, generally *melitensis* infections, characterized by step-like increases in the temperature from day to day to a maximum and, after a time, gradual decrease in temperature and possibly successive repetitions of this sequence of events; (4) the malignant type, almost always *melitensis* infections, in which the temperature is high and sustained with an extreme hyperpyrexia before death; and (5) an atypical chronic type which may take the form of muscular stiffness, gastric disturbances and various neurological symptoms. In general, undulant fever is a disease of relatively long duration, one to four months, and relapses during convalescence are not infrequent. In a chronic form undulant fever may be difficult to diagnose.

Brucellosis in man is a generalized infection as a rule, while in lower animals it is a localized infection, particularly in cattle. The bacilli may be isolated from the blood stream in man and the development of agglutinins is a diagnostic aid. Man also becomes sensitized to the cell substance of the bacteria, a hypersensitivity that is sometimes manifested as skin eruptions which may be macular or resemble the rose spots of typhoid fever. Localization may occur, however, and meningitis and meningo-encephalitis are probably not so rare as has been supposed, while in some instances orchitis, cholecystitis, endocarditis and other local manifestations have been reported. Pulmonary lesions with infiltration of the hilar glands or lung tissue proper are occasionally observed. Pulmonary infection has led many investigators to suspect infection by inhalation, and Elberg and Henderson¹³ have shown that the guinea pig may be experimentally infected by an intake of about 36 microorganisms inhaled as an aerosol. *Brucella* infection may be associated with abortion and mastitis in the human female in rare instances.¹⁴

¹² For a discussion of brucellosis in wildlife see the review by Katz: Jour. Amer. Vet. Med. Assn., 1941, 99:24.

¹³ Elberg and Henderson: Jour. Inf. Dis., 1948, 82:302.

¹⁴ The clinical aspects of human brucellosis are discussed in detail by Harris: *Brucellosis*. Paul B. Hoeber, New York, 1941; *ibid.* Jour. Amer. Med. Assn., 1946, 131:1485.

of the food without final cooking. The records make it clear that the danger from home-canned food is far greater than from that which is commercially prepared.

It should be noted that anaerobic conditions are not necessarily those in which the air is excluded either by a closed container or because the contaminated food is densely packed. Toxin can form, for example, in loose wet mincemeat exposed to the atmosphere (Aitken *et al.* 1936).

In pickled foods at least 2 per cent. of vinegar is necessary to destroy the spores, and in salted fish the salt content should be not less than 10 per cent. if growth of the organisms is to be prevented.

Given very favourable conditions, *Cl. botulinum* may produce detectable toxin within 12 hours, but usually it takes 2-14 days, depending on the temperature of storage of the food and other factors. According to Tanner and Oglesby (1936), and Tanner, Beamer and Rickher (1910), little or no growth occurs below 10° C., but food stored above this temperature may become dangerous fairly rapidly. The optimum temperature appears to be from 35-40° C. No growth occurs at 45° C. (Report 1953, Ohye and Scott 1953).

Diagnosis.

The symptoms of botulism in man are generally so characteristic as to render the disease capable of being diagnosed on clinical grounds. To confirm this diagnosis the following procedure should be adopted.

A. Demonstration of Botulinum Toxin in the Suspected Food.—For rapid diagnosis the food is suspended in saline, and injected intraperitoneally into three mice. A few hours before the injection, one mouse is given subcutaneously a small dose of antitoxin Type A and another Type B. Alternatively the food suspension may be added to the antitoxin, and the mixture injected subcutaneously. If the control mouse and one of the protected mice die, it may be provisionally concluded that *botulinum* toxin was present in the food, corresponding to the type of antitoxin which was given to the surviving mouse.

Meanwhile the food should be seeded into 2 per cent. glucose broth, Hitchens' medium (0.2 per cent. dextrose infusion broth containing 0.1 per cent. agar), beef heart peptic digest liver broth, peptonized bullock's heart broth (de Lavergne and Abel 1925), pork infusion thioglycollate semi-solid agar medium containing 0.1 per cent. of soluble starch (Wynne and Foster 1948), or cooked meat medium, and incubated anaerobically for 10 days at 35° C. The culture is then filtered, and the filtrate tested by intraperitoneal injection of mice or subcutaneous injection of guinea-pigs; antiserum Type A and B should be administered to two of the animals in order to determine the type of toxin present.

B. Isolation of the Bacillus from the Food.—Pure cultures may be obtained by heating broth cultures to 80° C. for half an hour to destroy contaminating non-sporing bacilli, and seeding into deep agar cultures, which are incubated anaerobically, preferably in the presence of added CO₂. The characteristic colonies should be picked off, grown in liquid medium, and tested for toxin production (Burke 1919b). Single colonies may likewise be obtained by plating the broth culture, after heating, on 5 per cent. horse blood extract agar plates incubated anaerobically (Wheeler and Humphreys 1924).

C. Demonstration of Botulinum Toxin in the Blood and Tissues.—Toxin may occasionally be demonstrated in the circulating blood during life. Schneider and

Epidemiology Brucellosis in man is probably always acquired from infected domestic animals, man-to-man transmission is a possibility but rarely, if ever, occurs. The commonest modes of infection in the United States are, first, the use of raw milk from infected cattle and, second, direct contact with the flesh of infected animals, both cattle and swine. As indicated above, animals may be readily infected via the alimentary tract, and it is not unreasonable to suppose that man is infected in this way also. The discharge of *Br. abortus* in the milk of infected cattle, then, provides the opportunity for infection when the milk is ingested in the raw state and in many instances undulant fever is acquired in this way. *Br. abortus* has been found in certified milk in a number of localities.¹⁵ The pasteurization of milk, of course, provides adequate protection from this source of infection.

The penetration of the unbroken skin by *Brucella* has been pointed out earlier. Man may be infected by the handling of the tissues of diseased animals or by close contact with other infectious material, presumably the bacilli enter through minute abrasions in the skin, or possibly through the intact skin. Employees of slaughterhouses, veterinarians, sausage-makers and butchers are, of course, particularly exposed to infection by this means and, in fact, the incidence of brucellosis in this group is disproportionately high. It is probable that most infections with *Br. suis* are acquired in this manner, although in some instances cattle are infected with this species and man acquires a *suis* infection via raw cow's milk.

Laboratory infections with *Brucella* are very common and even the most skilled workers have acquired undulant fever through working with these bacteria. Meyer and Eddie¹⁶ have reviewed 74 cases up to 1940 of which 44 occurred in competent bacteriologists. These infections are, in all probability, a consequence of handling infectious material and penetration of the skin by the microorganisms.

Undulant fever may also be acquired by drinking raw goat's milk, but infections with *Br. melitensis* from these animals are thought to be relatively infrequent in the United States. Evans,¹⁷ however, has found a number of cases in North Carolina, Kansas and Texas, and it is known to occur in the Southwest in general where goat's milk is consumed.

It has been shown experimentally that brucellosis may be transmitted by mosquitoes and biting flies, but at present there is no indication that this mode of transmission is of any significance in nature. Water is apparently not a vehicle of transmission; the single water-borne outbreak that has been reported¹⁸ was in the nature of a laboratory accident.

The prevalence of human brucellosis is not known with any degree of precision. As a consequence of better diagnosis, the number of reported cases has steadily increased from 24 in 1925 to 2497 in 1937. During the period 1930-1941 29,594 cases of brucellosis were reported in the United States, an average annual rate of 1.87 per 100,000 population.¹⁹ In 1945 a total of 44 states reported 4621 cases and 92 deaths, rates of 4.0 and 0.1

¹⁵ See, for example, Hasley: Jour. Inf. Dis., 1930, 46 430.

¹⁶ Meyer and Eddie: Jour. Inf. Dis., 19

¹⁷ Evans: Pub. Health Repts., 1937

¹⁸ Huddleson and Munger: Amer. J.

¹⁹ Jordan, Borts, Harris and Jenn

h., 1940, 30 944.

ub Health, 1943, 33 773

goats (Kempner 1897, Forssman 1905) and horses (Leuchs 1910) with each type of toxin. The antitoxin neutralizes the toxin according to the law of multiple proportions (Kempner 1897). Its therapeutic properties are limited. Injected before or simultaneously with the toxin it is protective for guinea-pigs and mice. Kempner (1897) found that 300,000 neutralizing doses, given 24 hours after the injection of the toxin, saved the life of a guinea-pig. Similar results were obtained by Kempner and Pollack (1897). Toxin-antitoxin mixtures given by the mouth are said to be dissociated in the stomach (Leuchs 1910). No international standard has yet been laid down for the antitoxin, but the intravenous inoculation of mice with toxin-antitoxin mixtures appears to be a promising method of titration (Glotowa and Dankerowitz 1935).

In the treatment of human cases antiserum has not yet been proved to be effective. Some favourable results have, however, been reported (McCasky 1919, Geiger 1920). Large doses, 50 ml. or more, of polyvalent serum, or of monotypical serum if the type of the intoxicating organism is known, should be given intravenously every day till the patient recovers, or all hope is abandoned. A prophylactic dose of 10 ml. should be given intramuscularly to all who have partaken of the poisonous food, and who have not yet developed symptoms of the disease. The antitoxin unit in the U.S.A. is that amount which prevents death within 4 days of guinea-pigs weighing 250 gm. injected with 1,000 M.L.D. of toxin.

Burke, Elder and Fischel (1921) state that liquid soap neutralizes the toxin, and that olive oil prevents its absorption from the gut. They advise therefore the use of high enemas of soap and olive oil. Iodine and potassium permanganate are both able to destroy the toxin *in vitro*, and might reasonably be given by the mouth. Alcohol precipitates the toxin (Armstrong *et al.* 1919); and it is possible that frequent small doses of brandy might prove beneficial. Mutch (1937) draws attention to the value of medicinal kaolin in the treatment of food poisoning on account of its ability to adsorb toxin. It is doubtful, however, whether any of these methods is likely to be of value in practice, since sufficient toxin will already have been absorbed by the time treatment is begun.

Botulism in Animals

Of recent years considerable attention has been devoted to a study of diseases of domestic animals characterized as a rule by fairly sudden onset, paralytic symptoms and often death. In many instances it has been possible to demonstrate a relationship between the type of fodder used and the occurrence of the outbreak. Organisms of the *botulinum* type have frequently been isolated from the animals and from the fodder, and the toxin produced in culture has been shown to be capable of reproducing the symptoms of the disease when given by the mouth or inoculated subcutaneously. The complete chain of evidence necessary to incriminate these organisms in the causation of the disease has often been lacking, but there seems to be little question that in many outbreaks the diagnosis of botulism has been essentially correct.

Great confusion exists about the exact identity of the various organisms isolated. Because some of them differ in their toxin production from the classical *Cl. botulinum* A or B types, the names *Cl. parobotulinum*, *Cl. parobotulinum bovis*, or *Cl. parobotulinum equi* have been suggested, and the disease caused by them has been termed parabolism. This is not the place to discuss bacteriological nomenclature, but we are in entire agreement with Weinberg and Ginsbourg (1927) that

Forage poisoning is a wide term covering a number of different conditions, and it is not to be thought that it is uniformly due to poisoning with the toxin of *Cl. botulinum*. According to Walker (1929), the grass disease of this country is almost certainly different from botulism; and according to Gordon (1934) a disease in some respects resembling grass sickness may result from intoxication with products of *Cl. welchii* (see Chapter 36). In Belgium Willems (1941) showed that the *Mal d'Aiseau*, which was thought to be a form of Borna's disease, was in fact due to *Cl. botulinum* Type D. The same organism is apparently responsible for the widespread enzoötic of botulism among horses in France (Prévot and Brygoo 1950).

Botulism in Cattle and Sheep.—Forage poisoning in cattle is commonest between the ages of 6 months and 2 years; the case-fatality rate is 2–10 per cent. (Graham and Schwarze 1921b). From a corn silage that was shown to have been responsible for an outbreak in cattle on two different occasions, Graham and Schwarze (1921b) isolated *Cl. botulinum* Type B. Whether the organism had any causal relationship to the poisoning was not definitely ascertained.

Dubovsky and Meyer (1922a) isolated *Cl. botulinum* from the liver and mesenteric glands of two cows suffering from ictero-hæmoglobinuria. In Australia Seddon (1922) investigated an epizootic disease of cattle known by the names of Midland cattle disease, impaction paralysis, or dry bible; this disease appears to be identical with the *lamziekte* of South Africa. From animals dying of the disease he isolated an organism, which he called *B. parabotulinus*; this organism is now referred to as the C_p type of *Cl. botulinum*. Experimentally its toxin gave rise to bulbar paralysis in cattle.

Our knowledge of botulism in cattle was extended by Theiler and his colleagues (1926–27) working on *lamziekte* in South Africa. This disease, which is characterized by paresis and paralysis, principally of the locomotor system, but sometimes of the muscles of mastication and deglutition, occurs in areas where the phosphorus content of the soil, and hence of the pasturage, is deficient. According to Theiler, the affected animals have a craving for phosphorus. This they endeavour to satisfy by eating the debris of carcasses, especially bones, which they find on the veld. Some of these carcasses, or the remains of muscle around the bones, happen to be infected with *Cl. botulinum*, and if sufficient toxin has been produced by the multiplication of this organism, the animals are liable to develop the characteristic symptoms of *lamziekte* (see also Seddon 1927). The disease can be prevented by feeding bone-meal to the animals; this satisfies their craving for phosphorus, and so prevents their indulging in the so-called practice of "osteophagia." The infecting organism appears to be mainly of the D type, but, as already noted, it is closely related to the C_p type found in Australia by Seddon. According to Scheuber (1929), it is found in the soil near decomposing carcasses, and often in the intestinal contents of both sick and healthy animals.

Bennetts and Hall (1938) found that botulism of cattle and sheep in Western Australia resulted largely from the eating of carrion containing the toxin of Type C. Experimentally, they were able to show that as little as 3–5 gm. of toxic rabbit carrion might prove fatal to sheep. The length of time that carrion remained toxic varied with the rainfall and other factors, but sometimes it might be as long as six months. Vaccination with two doses of alum-precipitated *botulinum* toxoid at an interval of not less than two months conferred on both cattle and sheep

Immunity.²¹ The resistance of calves and non pregnant cows to clinically apparent brucellosis is clearly an expression of natural immunity, though the older animals respond to the microorganism with the production of antibodies and the development of an increased resistance to subsequent infection. Man likewise appears to have a high degree of natural resistance to the infection and it is probable that there are many more infections than clinical cases of brucellosis. In the series studied by Huddleson and Munger²⁴ in which exposure to infection was known, only about half the individuals showing evidence of infection by an immune response had clinically apparent disease.

In man the immune response is evidenced by the appearance of agglutinins, opsonins and hypersensitivity to preparations (*brucellergen*) of the cell substance of the bacteria. It is not clear, however, that this response is associated with an increased resistance, i.e., effective immunity, to the infection. Prophylactic inoculation with *Brucella* vaccines is not practical in man, though the available evidence indicates that it is effective in cattle. Killed vaccines have not given satisfactory results, but those of living avirulent bacilli have given encouraging results in the control of bovine brucellosis by vaccination of calves. A smooth, avirulent strain known as strain No. 19 has been the most widely used. The desirability of general vaccination is not completely agreed upon, however, since it does not eliminate infection in herds.²² The therapeutic use of antisera or vaccines in human brucellosis has given disappointing results.

BRUCELLA BRONCHISEPTICA

This microorganism is very similar to *Br. abortus*, but is motile and highly aerobic. It does not produce hydrogen sulfide. It is immunologically related to *Br. melitensis* and *Br. abortus*, but can be separated from them by agglutinin-absorption tests. It also resembles *Hemophilus pertussis* both culturally and immunologically. Originally isolated from dogs ill with distemper, it is not now generally believed to stand in any causal relation to that disease. It is, however, frequently found as the cause of bronchopneumonia in guinea pigs and other rodents.

²² See the discussions by Dykstra Jour. Amer. Vet. Med. Assn., 1947, 110.96; Crawford *ibid.*, 1947, 110.99; Haring *ibid.*, 1947, 110.103.

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PASTEURELLA

Hemorrhagic Septicemia; Plague; Tularemia

The term "hemorrhagic septicemia" was applied by Hueppe to a group of highly fatal infectious diseases of the lower animals in which large and small hemorrhagic areas are found in the subcutaneous tissues, serous membranes, muscles and lymph glands, and throughout the internal organs. The causative bacteria constitute a group of closely related, biochemically inactive, non-motile, gram-negative forms showing bipolar staining. The first of these bacteria to be studied was the etiological agent of fowl cholera which Pasteur used in his early studies on immunity. Others have been described as producing hemorrhagic septicemias in various lower animals. Regarded by some as but a single species under the name *Pasteurella pluri-septica*, these bacteria are usually separated into species which differ from one another in host adaptation and minor fermentation reactions. They have been given names derived from the kind of animal in which they were found.

The species of bacteria causing the hemorrhagic septicemias of lower animals are:

Pasteurella aviseptica (*Bacillus avisepticus*, *Bacterium avisepticum*, *Pasteurella avicida*)—the fowl cholera bacillus. Pathogenic for birds and mammals.

Pasteurella muriseptica (*Bacillus murisepticus*, *Bacterium murisepticum*, *Pasteurella muricida*)—found in naturally infected wild rats and pathogenic for rabbits, guinea pigs, mice and rats but not for chickens or pigeons.

Pasteurella leipseptica (*Bacillus leipsepticus*, *Bacterium leipsepticum*, *Pasteurella cuniculicida*)—the bacillus occurring in contagious nasal catarrh or "snuffles" of rabbits as a clinical or latent infection. Produces septicemia in rabbits upon parenteral inoculation. Pathogenic for chickens and mammals.

Pasteurella suis-septica (*Bacillus suis-septicus*, *Bacterium suis-septicum*, *Pasteurella suilla*)—the bacillus of swine plague. Pathogenic for mice, rabbits and birds.

Pasteurella bovis-septica (*Bacillus bovis-septicus*, *Bacterium bovis-septicum*, *Pasteurella bollingeri*)—produces a hemorrhagic septicemia in cattle, hogs and horses and is found in deer and wild hogs.

So far as is known, the diseases produced by these bacteria are not ordi-

narly communicable to man. Rare cases of human infection with bacilli of this group have, however, been reported.¹ Closely related to this group and classified as species of *Pasteurella* are the bacilli of plague or "black death" and of tularemia or "rabbit fever." *Past. pestis* differs culturally from the hemorrhagic septicemia bacilli in that it grows in the presence of bile, does not ferment sorbitol, and does not produce indol or hydrogen sulfide. Unlike the other *Pasteurella* species, *Past. tularensis* requires enriched media for growth.

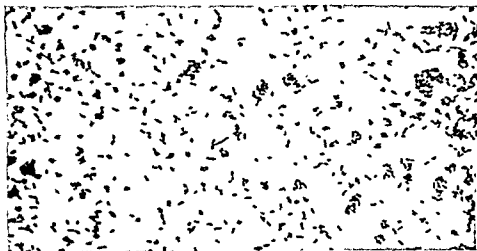


Fig. 92. *Pasteurella suisseptica*. Smear from a pure culture. Fuchsin, $\times 1050$.

PASTEURELLA PESTIS—THE PLAGUE BACILLUS

Plague prevailed extensively throughout Europe during the Middle Ages. It has been estimated that 25,000,000 persons, or one-quarter of all inhabitants of Europe, perished in the "great mortality" or "black death" of the fourteenth century (1348-49). Few diseases have left so deep a mark on general literature. Boccaccio's *Decameron* contains one of the most vivid descriptions of the plague ever written, and Defoe's fictitious² *Journal of the Plague Year* provides a realistic picture of the devastation of London in 1665 by an outbreak of "black death" in which 70,000 persons perished.

For reasons that may be only partly conjectured the plague has had irregular periods of quiescence and recrudescence. Western Europe has been practically free from the plague since the middle of the eighteenth century, and the disease began its first great extension in modern times with its appearance in 1893 in Hongkong and in 1896 in Bombay. During recent years the plague has caused terrible loss of life in British India; official statistics show that in the period from 1896 to 1918 more than 10,000,000 deaths were due to this disease. In October, 1899, a case was recorded at Santos, Brazil, this is thought to be the first occurrence of the plague in the Western Hemisphere. Plague first appeared in the United States in San Francisco in 1900, it is assumed that it was introduced by infected rats from

¹ The hemorrhagic septicemia bacilli are discussed at length by Regamey. *Les infections humaines a B. bipolaris septicus (Pasteurelloses)*. Huber, Berne, 1939.

² Defoe was only four years old in the year of the great plague.

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the Orient. The infection apparently spread to ground squirrels and other wild rodents in the western part of the country.

The plague bacillus, *Pasteurella pestis*, was discovered almost simultaneously by Yersin and by Kitasato in 1894.

Morphology and Staining. The plague bacillus is a short, plump, ovoid rod 0.3 to 1.25 μ in length. In the body fluids the bacilli may occur in pairs, but long chains are rare and, in general, there is no characteristic arrangement. The bacilli are non-motile and are encapsulated. Involution forms are common, especially in older cultures, and coccus shapes, large rods and gigantic swollen forms may be observed. The tendency of the plague bacillus to aberrant morphology is accentuated by cultivation on media containing 3 to 4 per cent sodium chloride; the appearance of involution forms in twenty-

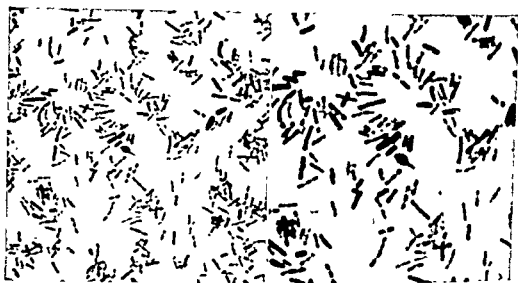


Fig 93. The plague bacillus Smear from pure culture; fixed in methyl alcohol and stained with methylene blue to show bipolar staining. Note the involution forms present even at twenty-four hours' incubation. Left, $\times 1050$, right, $\times 1800$.

four-hour cultures on salt-containing media has been regarded by some as a characteristic of differential value.

Colonies on nutrient agar or gelatin have a delicate, drop-like appearance, with a round, granular center and a thin, granular, uneven margin.

The plague bacillus is uniformly gram-negative and shows a marked tendency toward polar staining, i.e., there are heavily stained areas at the ends of the cell separated by a lightly stained area in the center (see Fig 93). For good bipolar staining the smear should be air-dried and fixed in alcohol. The usual aniline dyes, such as methylene blue, are satisfactory. The plague bacillus is best demonstrated in tissue sections by a polychrome stain.

Physiology. *Past. pestis* is not nutritionally fastidious, and growth occurs on all the ordinary culture media, although in peptone water it is very poor. Some strains will grow in amino acid media without bacterial vitamins while others require nicotinamide or thiamine or both.³ Unlike most of the bacteria pathogenic for man, a temperature of 25° to 30° C.

³ Berkman. Jour. Inf. Dis., 1943, 71:201; Doudoroff Proc. Soc. Exp. Biol. Med., 1943, 53:73.

CHAPTER 73

PLAGUE, PASTEURELLOSIS, AND PSEUDOTUBERCULOSIS

PLAGUE

INTRODUCTORY

PLAGUE has been one of the greatest scourges of the human race. From time to time it has swept over the world in relentless waves, exacting a toll of life probably unequalled by that of any other epidemic disease. In the 5th and 6th chapters of the 1st Book of Samuel there is an unmistakable account of bubonic plague (see MacArthur 1952). Before the Christian era 41 epidemics are on record, during the 1,500 years following the birth of Christ there are records of 109 epidemics, including the great plague of Justinian's reign, and the Black Death of the fourteenth century. Between 1500 and 1720 there were 45 pandemics of plague (Editorial 1925a). During the eighteenth and the nineteenth centuries it was comparatively quiescent, being confined almost entirely to endemic foci in various parts of Asia; but at the close of the last century it sprang once more into activity. Starting in Hong-Kong in 1894, it invaded India, Japan, Asiatic Turkey, and European Russia in 1896; the following year it reached Madagascar and Mauritius. In 1899 Arabia, Persia, the Straits Settlements, Austria, Portugal, British South Africa, Egypt, the French Ivory Coast, Portuguese Africa, the Argentine, Brazil, Paraguay, and the Hawaiian Islands were affected. There was a small outbreak at Glasgow in 1900, and in the same year the disease appeared at Sydney in Australia and at San Francisco, California. Undoubtedly the brunt of the disease was borne by India, where in the 20 years 1898-1918 more than 10 million deaths were recorded (White 1918-19). (For general review of history and of present prevalence, see Hoekenga 1947, Kaul 1949, Pollitzer 1954.) At the present time bubonic plague is disseminated widely but unevenly throughout the world, foci are present in India and South-East Asia, in parts of Africa and in South America (Kaul 1949).

One of the most disturbing features is the progressive infection of rodents that is occurring in the Americas and in South Africa, constituting what is known as rural, wild rodent, or sylvatic plague in contradistinction to bubonic plague, which is spread by rats.

The causative organism was isolated independently and almost simultaneously by Kitasato (1894) and by Yersin (1894), at Hong-Kong in 1894. Numerous workers, particularly Ogata (1897), Simond (1898), and Gauthier and Raynaud (1902, 1903) were responsible for showing that the disease is primarily one of rodents, and that it is spread to man by the agency of infected fleas. This conception was criticized by several workers (Nuttall 1898, Galli-Valerio 1900, 1903), but was definitively proved to be correct by the English Plague Commission (Report 1906).

is more favorable than one of 37° C., and the limiting temperatures for growth are -2° C. and 45° C. In any case, the colonies on solid media grow slowly and never attain a large size. The plague bacillus is aerobic and facultatively anaerobic.

Sugar fermentations are variable, and a small amount of acid but no gas is produced. Neither coagulated serum nor gelatin is liquefied, and indol is not produced. Nitrates are reduced to nitrites, and a small amount of hydrogen sulfide is formed. On potato and in milk multiplication is slow and scanty, milk is rendered slightly acid but is not curdled.

One of the most characteristic cultural features is observed in the growth in broth. When the surface of this medium is covered with a layer of oil and flasks are left undisturbed for five or six days after inoculation, long, delicate filaments are formed which hang down from the surface into the depths of the clear broth, like the stalactites that depend from the roof of a grotto. Not all cultures of *Past. pestis* show stalactite growth in equal degree, and, on the other hand, a similar formation has been observed in cultures of other bacteria, the stalactite formation, therefore, while highly characteristic, especially when broth is seeded directly from fresh plague buboes, is not specific.

The plague bacillus does not exhibit any marked resistance to deleterious influences. Exposure to drying, particularly at the higher summer temperatures, kills it within a short time. The bacillus is quite sensitive to the action of sunlight and chemical disinfectants, it is killed, for example, by 0.5 per cent phenol in ten to fifteen minutes and by heating to 55° C. in about the same time. Cultures kept in the refrigerator, however, remain viable over long periods of time. In general, the life of *Past. pestis* outside the animal body is precarious, and the bacillus seems to disappear speedily from soil, water and buried cadavers.

Toxins. The toxicity of old broth cultures to experimental animals on parenteral inoculation is indicative of the toxicity of the bacillary cell substance. Baker *et al.*⁴ have isolated an endotoxin by chemical fractionation of the bacilli which had an LD₅₀ dose for mice of 0.6 γ and was not identical with an immunizing protein antigen. The plague bacillus also contains a factor which enhances spreading in the tissues and increases capillary permeability, and a coagulase is produced.⁵

Pathogenicity for Man. Plague in man appears most commonly in two forms, the bubonic or glandular plague and plague pneumonia. In the bubonic type the symptom-complex is characteristic, and diagnosis on clinical grounds is relatively simple. From the buboes, which may be either primary or secondary, bacilli may pass over into the blood; in fatal cases the bacteria often multiply in the blood extensively. The case fatality is 60 to 90 per cent. A primary plague septicemia can also probably occur. There are sometimes subcutaneous hemorrhages. During the plague epidemics in the Middle Ages such hemorrhages seem to have been more frequent than at present, and the dark spots to which they give rise were the origin of the popular name of "black death."

Plague pneumonia occurs secondary to the glandular infection and may

⁴ Baker *et al.*: *Proc. Soc. Exp. Biol. Med.*, 1947, 64:139.

⁵ Jawetz and Meyer: *Jour. Immunol.*, 1944, 49:15.

The case fatality of bubonic plague is about 60-90 per cent., of pneumonic plague 100 per cent.

In Bombay the English Plague Commission (Report 1907, p. 724) determined that prior to the annual outbreak of human plague there was an epizootic amongst the rats. First the rats belonging to the species *Rattus norvegicus* (*Mus decumanus*) were affected. After an interval of about 10 days an epizootic appeared in the rats of the species *Rattus rattus* (*Mus rattus*); and after a further interval of 10 days the human epidemic broke out. From this they inferred that *R. rattus* was infected from *R. norvegicus*, and subsequently conveyed the disease to man. It was further observed that plague persisted in *R. norvegicus* during the off-season—in Bombay from June to December—and flared up at the onset of the colder weather (Fig. 288, p. 1837).

The transference of plague from rat to rat and from rat to man occurs almost exclusively by fleas. Chief amongst these are *Xenopsylla cheopis* and *Ceratophyllus fasciatus*—the rat fleas. It has been shown that in the absence of their specific hosts, both of these types of flea will bite human beings. Plague is essentially a septicæmic disease; towards the end of an attack the bacilli are present in the blood in enormous numbers, and are readily imbibed by the fleas that infest the rat. When the animal dies, the fleas leave the corpse and wait for a suitable opportunity to attach themselves to a fresh host. Meanwhile the bacilli multiply in the proventriculus, often to such an extent as to block it completely, and prevent access of food to the stomach. A flea in this condition is hungry, and when it succeeds in finding a new host, attacks it with vigour. The act of sucking, however, only distends the already contaminated œsophagus, and on the cessation of the pumping act some of the blood is forced back into the wound (Bacot and Martin, see Report 1914). Sometimes a temporary passage is cleared through the mass of obstructing bacilli; this fails, however, to restore the lost valvular function to the proventriculus; it merely leaves a passage through which the blood can flow out of the stomach as freely as it enters. Hence after a full meal, blood extends from the posterior portion of the stomach to the anterior chamber of the pharyngeal pump. Such a flea is probably more dangerous than one whose proventriculus is completely blocked, since the contents of the stomach can be regurgitated into the wound with greater freedom (Report 1915b, Burroughs 1947).

The length of time that a flea can remain infected depends on several factors, chief of which are temperature and humidity. In India fleas were found to harbour plague bacilli up to 47 days (Report 1915a), in Madagascar for several weeks (Girard 1936), and in the United States for as long as 130 days (Eskey and Haas 1940). Survival of the bacilli in the flea is favoured by a low temperature—about 50° F.—and a nearly saturated atmosphere; adverse factors are a temperature over 80° F., or an even lower temperature with a dry atmosphere (Liston 1924). But the time that fleas can remain infective is much shorter; most of them die in a day or two, and the limit of infectivity is probably about 7-14 days.

It has been suggested that the bacilli in the flea's stomach may be attacked by the phagocytes in the rat's blood which has been ingested; when the temperature rises the phagocytes become more active. This may explain why a flea clears itself more quickly at a high than at a low temperature, and also why, if it is fed on healthy blood after being infected, it clears itself more quickly than when it is starved. The clearance is said to be still more rapid when the flea is fed

fected insect vectors (as differentiated from mechanical transmission by insects), the gut of most blood-sucking insects is strongly bactericidal and bacteria do not persist there more than a few hours. *Past. pestis* and *Past. tularensis*, however, are resistant to this bactericidal activity and the insect vectors may remain infective for days and perhaps weeks.

Plague is also present in other rodents. In California the native ground squirrels have proved highly susceptible to infection, and through the agency of these animals a great reservoir of infection termed *sylvatic plague* has developed.⁷ The infection may be overt and occur in epizootic form, and it may also persist as an inapparent latent infection.⁸ The squirrel

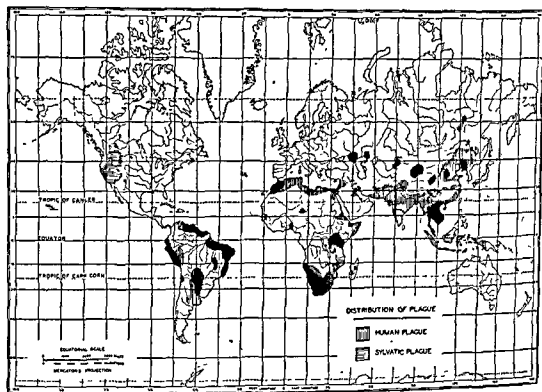


Fig. 96. The world-wide distribution of human and sylvatic plague. Redrawn from map prepared by Army Medical Intelligence, 1943. (Based on Goode Base Map. No. 201M. By permission of the University of Chicago Press.)

flea, *Ceratophyllus acutus*, transmits the infection to man. In other parts of the world other rodents are affected and are the means by which the disease is maintained and spread. In South Africa it is the gerbille, in Transbaikalia the tarbagan, and in Russia the spermophile that keeps the plague alive in animal reservoirs of infection; there is a focus of sylvatic plague on the Peruvian-Ecuadorian frontier in wild rodents. The tendency of the plague bacillus to establish these reservoirs in wild rodents constitutes a danger not fully appreciated. There is reason to believe that rodent infection is extending in several parts of the world, and there is also some experimental evidence that plague infection of man contracted from wild rodents is especially likely to assume the deadly pneumonic form.

⁷ Plague in the western part of the United States is considered in detail by Eskey and Haas. *Pub. Health Bull.* No. 254, 1940.

⁸ Meyer *et al.*: *Jour. Inf. Dis.*, 1943, 73:144.

on the blood of immunized animals (Report 1908, p. 260). It is possible that in this manner the increasing proportion of immune rats towards the close of an epidemic exercises an inhibitory effect on the spread of the disease.

Plague in Rats.

In India the two species mentioned above are chiefly responsible for the spread of plague. *Rattus norvegicus* is the large grey rat; it lives in sewers, stables, and garbage. *Rattus rattus* is the black rat; it is smaller and less fierce than the grey rat, and, living in houses or their immediate neighbourhood, it comes into closer contact with the human population. The natural mode of infection of rats appears to be almost entirely by fleas. It is possible that animals may acquire the disease by devouring their dead companions; but considering that it is not easy to infect rats by feeding them on plague material, and that the post-mortem appearances in rats dying naturally of plague differ from those of rats experimentally infected by feeding, it is doubtful whether this is more than a rare occurrence.

In Bombay, of the dead rats examined during 1905-06, 22.2 per cent. of *R. norvegicus*, and 16.7 per cent. of *R. rattus* were infected with plague. Of the live rats the figures were 0.85 per cent. and 0.37 per cent. respectively. Thus plague was commoner in *R. norvegicus* than in *R. rattus* (Report 1907, p. 724). The susceptibility of the two species to experimental inoculation with plague was almost identical. In Sydney during 2½ years ending December 31, 1904, 125,872 rats and mice were caught and examined; plague was identified in 0.37 per cent. (Thompson 1906).

During the height of the epizootic the lesions are those of acute plague, but during and subsequent to the decline, a number of healthy rats are encountered with atypical lesions. One of the commonest of these is a large abscess in the spleen or liver, containing plague bacilli. The rats, though infected, have evidently been able to withstand the attack. Swellengrebel and Hoesen (1915) in Java, and Bordas, Dubief and Tanon (1922) in France found a number of rats with atypical lesions containing plague bacilli of lowered virulence; on passage through one or two guinea-pigs they became fully virulent again. Williams and Kemmerer (1923) in New Orleans confirmed the existence of *latent plague* in rats. It has also been found among other rodents in Russia and the United States. Whether these animals act as chronic carriers of the disease and are responsible for keeping alive the infection from one epizootic to the next is still an open question. Most workers are disposed to attribute more weight in this respect to the survival of plague bacilli in the flea than in its host (Girard 1936, Eskey and Haas 1940).

Experimental Transmission of Plague in Rats.—It has been abundantly demonstrated by different workers that plague does not spread from rat to rat by contact in the absence of fleas. On the other hand infection spreads readily in the presence of fleas, even though the animals have no immediate contact with each other. Two experiments of the English Plague Commission will illustrate this.

EXPERIMENT 1.—(Report 1910.) Bombay rats were inoculated with plague and mixed with normal rats in godowns. In one set of godowns fleas were excluded, but in the other set they were permitted to multiply. In the flea-free godowns none of the normal rats, which were in continuous contact with the infected rats, developed plague, whereas in the others infection spread rapidly (Table 141).

The rats dying of plague in the flea godowns nearly all showed cervical buboes,

As pointed out above, plague may assume two epidemiological forms. As pneumonic plague it is transmissible by droplet infection and its epidemiological behavior is very similar to that of the other respiratory infections. As bubonic plague it is derived from an infected rodent through the agency of the flea. In its epidemic form, bubonic plague is nearly always associated with the disease in the black rat. Infections derived from other rodents, such as the California ground squirrels, are sporadic rather than epidemic. The dissemination of bubonic plague in epidemic form, then, is determined by the closeness of the association between the infected rat population and the human population. Filth and poverty associated with a large rat population provide opportunities for transmission of the disease, and if transmission takes place on a large scale an epidemic may flare up and perhaps extend to even greater proportions as the pneumonic form develops.

Bacteriological Diagnosis of Plague. In man the bacilli are found in material aspirated from buboes, in cultures or smears of internal organs, especially spleen, and, in pneumonic plague, in the sputum. The presence of gram-negative, bipolar staining, ovoid bacilli is highly suggestive. Blood cultures, taken late in the disease, should be cultured first in broth. Other material may be inoculated directly on blood agar and glycerol agar. Cultures may be identified by cultural and biochemical characteristics and by agglutination in plague antiserum. The bacilli show some tendency to spontaneous agglutination and the slide agglutination test is unsatisfactory. Guinea pigs may be inoculated subcutaneously or, with specimens that have undergone gross contamination and decomposition, by rubbing the material on the freshly shaven abdomen, the plague bacilli penetrate the minute abrasions while the contaminants do not. The animals die in two to five days, postmortem findings are characteristic and include subcutaneous and general congestion, congested spleen, granular liver and pleural effusion. The bacilli may be found in spleen smears and elsewhere and cultured. It is important that the animal be freed of ectoparasites before inoculation. Plague in rodents may be diagnosed by postmortem findings, which are similar to those in the guinea pig, by microscopic and cultural demonstration of the bacilli, and by guinea pig inoculation.

Immunity. Recovery from plague confers a solid immunity to subsequent infection. Experimental animals may be immunized by inoculation with suspensions of attenuated or killed plague bacilli, and numerous attempts have been made to actively immunize man in this way. One of the first plague vaccines was that of Haffkine and consisted of heat-killed bacilli from old cultures. This and other killed vaccines have never been particularly satisfactory and do not produce an efficacious immunity in man. The vaccine used by the United States Army during World War II consisted of a suspension of 2000 million formalin killed virulent plague bacilli per ml., and was given in two doses, 0.5 ml. and 1.0 ml., seven to ten days apart. Inoculation with living attenuated bacilli produces a much more solid immunity in experimental animals, however, and avirulent strains appear to be as efficient immunizing antigens as virulent strains.⁸ The use of living attenuated cultures for human inoculation has been attempted in the past

⁸ Pirie and Grasset. *South African Med. Jour.*, 1938, 12:294, *ibid.*, 1941, 15:275.

of 6 godowns were specially constructed by the Commission. The walls were of brick and mortar, 9 inches thick; the floors were of concrete on top of a high plinth. Each godown measured internally 7 ft. \times 6 ft. Leading into the interior were double doors, lined with wire netting, between which was an inspection chamber. The essential difference in the structure of the godowns was in the roofs. Nos. 1 and 2 were roofed with country tiles, placed in four layers on the top of wooden laths. On the inside of this roof there was a wire netting on a wooden framework in Godown 2, and two layers of wire netting, 10 inches apart, in No. 1, so that while rats could build their nests in the tiles of the roof, they were completely shut off from the interior of the godowns. Mangalore tiles were used for roofing Godowns 3 and 4; these do not afford so good a shelter for rats as country tiles; a single layer of wire netting separated the tiles from the interior. Godowns 5 and 6 were roofed with a single layer of corrugated iron fastened down with cement to the tops

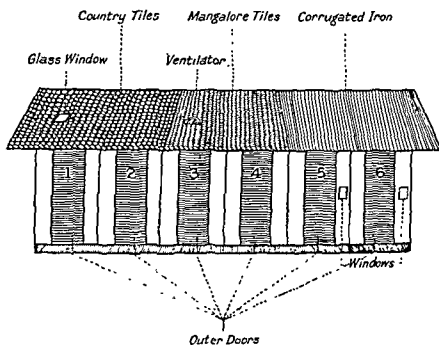


Fig. 290.

The doors opened into the left-hand side of each godown, not in the middle as indicated in the figure.

of the walls, so that no rats could nest at all. Godowns Nos. 1 and 3 had a small roof light; the rest were in darkness (Fig. 290).

By allowing 3 guinea-pigs to run about in each godown for 6 days, and making flea counts every day, it was ascertained that the number of fleas varied in each instance with the accessibility of the roof to rats. Thus in No. 1, 54 fleas; in No. 2, 228; in No. 3, 40; and in No. 4, 70 fleas were caught in 6 days. In Nos. 5 and 6 fleas were few or absent. Incidentally this experiment brings out the importance of darkness in favouring infestation by fleas.

In these godowns a series of experimental epidemics was initiated amongst guinea-pigs, generally by introducing a number of animals experimentally infected with plague and adding normal animals to them. For the detailed results, reference must be made to the Commission's Report (1906); the conclusions that were drawn from them were as follows:

(1) Close contact of plague-infected with healthy animals, if fleas were excluded, did not lead to the production of an epizootic. Since the godowns were never

but not continued because of possible attendant danger. The use of such vaccines in man has been investigated by Grasset¹⁰ in South Africa with encouraging results, and a similar vaccine has been used in recent years in the Netherlands Indies in more than ten million inoculations without untoward results.¹¹

Antisera to the plague bacillus may be prepared by the judicious immunization of horses, or other animals, but their efficacy as therapeutic agents is doubtful.

PASTEURELLA PSEUDOTUBERCULOSIS

Past. pseudotuberculosis (*Bacillus pseudotuberculosis rodentium*) causes a disease of rodents, particularly guinea pigs. It resembles *Past. pestis* very closely but, unlike the latter, is usually actively motile at 22° C. Other differential marks are its tendency to produce alkali in milk cultures and its relatively low pathogenicity for white rats. The natural mode of infection is probably by way of the alimentary tract. Subcutaneous inoculation of guinea pigs proves fatal in two to three weeks, with caseous swellings and nodules ("pseudotubercles," which have unfortunately given this bacterium its name) in various organs. *Past. pseudotuberculosis* has been found, though rarely, in animals other than the guinea pig, and a few cases of infection have been reported in man.

PASTEURELLA TULARENSIS

Tularemia is a disease of rodents, rabbits in particular, that is transmitted to man either directly through the handling of the flesh of infected animals or indirectly through an insect vector. *Past. tularensis* (*Bacterium tularense*) was discovered by McCoy and Chapin¹² in a plague-like disease of the California ground squirrel. Tularemia in man, however, is contracted largely from the rabbit and it was shown by Francis¹³ that the disease known in Utah as deer fly fever is, in fact, tularemia transmitted from infected rabbits to man by the bite of the fly *Chrysops discalis*. Francis also found that *Past. tularensis* was present in rabbits sold in the markets of Washington, D. C., and that a disease known as rabbit fever was not infrequent among those in contact with the rabbits. In 1938 2088 cases with 139 deaths and in 1939 2200 cases with 150 deaths were reported. Human cases have been observed in all forty-eight states and in the District of Columbia.¹⁴

Morphology and Staining. In culture *Past. tularensis* is a minute, gram-negative pleomorphic rod 0.2 μ in breadth and 0.3 to 0.7 μ in length, the coccoid form predominates in young cultures and the bacillary form in older cultures. In smears from the spleens of infected mice or guinea pigs the bacteria appear as coccoid forms in well-defined clusters. Capsules are present in the body; spores are not formed, and the microorganisms are

¹⁰ Grasset: Trans. Roy. Soc. Trop. Med. Hyg., 1946, 40:275.

¹¹ Cf. Otten: Mededeel Dienst Volksgezondheid Nederland-Indie, 1941, 30 61.

¹² McCoy and Chapin: Jour. Inf. Dis., 1912, 10 61.

¹³ Francis: Pub. Health Repts., 1919, 34:2061; *ibid.*, 1923, 38 1391, 1396.

¹⁴ Cf. Pub. Health Repts., 1940, 55:667.

by a layer of sand. When left in plague-infected houses, 24 per cent. of the animals in the unprotected cages developed plague whereas not one of the animals in the protected cages did so.

Mode of Spread of Plague in Bombay.—The conclusions drawn from these experiments receive support from epidemiological observations in the field. Plague in man predominates amongst the lowest classes, particularly those in dirty and insanitary dwellings, where rats and fleas are numerous. The higher classes, such as the Parsees and the Europeans, suffer very slightly. The presence of an initial bubo renders it highly probable that infection occurs by the skin, and the predominant selection of the inguinal region for the development of the bubo suggests that the bacilli gain entrance through the skin of the leg. In confirmation of this it has been noted by several observers that in about 5 per cent. of cases the first sign of plague is a small vesicle, surrounded by an area of redness (Simond 1898). This is found generally on the leg, and is undoubtedly due to the bite of a flea. Living plague bacilli are said to be uniformly present in these vesicles.

Though rats in Bombay breed throughout the year, both *R. rattus* and *R. norvegicus* breed most freely from June to October. The largest proportion of young rats in relation to the total population is reached in November and December.

Rat fleas are most prevalent during the months of February, March, April, and May. The infestation of *R. norvegicus* is more than double that of *R. rattus*. Thus the number of fleas per rat during these months is about 5 for *R. rattus* and 12 for *R. norvegicus*. In this connection it will be remembered that plague is commoner in *R. norvegicus* than in *R. rattus*. The epidemic season for rats and man lasts from January or February till April or May, the maximum prevalence being either in March or April. The sequence of events would appear to be as follows. During the hot season the rats breed freely, and a large susceptible population is produced. As the colder weather arrives, the fleas increase in numbers, and plague breaks out in *R. norvegicus*. This is rapidly followed by an epizootic in *R. rattus*, and this in its turn by the human epidemic. At the onset of the hot weather in May or June the flea population decreases rapidly, and plague in rats and man comes to an end for the year. The subsidence of the epidemic is accounted for partly by the increasing proportion of immune rats, and partly by the decrease in the number of fleas. With regard to the former it was found by the English Commission that Bombay rats captured towards the end of the epizootic were more resistant to experimental inoculation with *Past. pestis* than those at the beginning. Whether the rats that had survived the epizootic were more resistant *ab initio*, or had acquired immunity during the progress of the epizootic, it is impossible to say.

During the off-season sporadic plague occurs in rats, particularly *R. norvegicus*, and in man; but the conditions are obviously unsuitable for its spread on any but a small scale.

We have dealt at some length with the conditions prevailing in Bombay, because they have been investigated so thoroughly. In other parts of India much the same sequence of events has been observed, with, however, variations dependent on climatic and other factors. It is curious that 75 per cent. of the plague cases in India are distributed over the Punjab, the Bombay Presidency, and the United Provinces, when it is remembered that these areas contain less than a third of

non-motile. According to Hesselbrock and Foshay¹⁵ it reproduces by a number of methods, including binary fission, budding, filament formation and the like, and they regard it as closely related to the microorganisms of the pleuropneumonia group (p. 547).

On solid media *Past. tularensis* forms minute, transparent, droplike colonies that are mucoid in consistency and readily emulsifiable.

This bacterium is somewhat difficult to stain, methylene blue is not satisfactory, but either carbol fuchsin or aniline gentian violet may be used. Bipolar staining may be observed.

Physiology. *Past. tularensis* differs sharply from the other members of this genus in that it will not grow on the ordinary media. It may be cultured on a coagulated egg-yolk medium or on blood dextrose cystine agar. Until recently it has been assumed that this bacterium could not be grown on a liquid medium Tamura and Gibby¹⁶ have found, however, that



Fig 97, *Pasteurella tularensis*. Note change from coccoidal to bacillary form in twenty-four hours on fresh culture medium (Francis).

growth occurs in casein or gelatin hydrolysate medium supplemented with biotin, blood cell extract and liver extract. Steinhaus and McKee¹⁷ have found that a medium of heart infusion, dextrose, cystine and hemoglobin will support good growth. It is aerobic and facultatively anaerobic, and its optimum temperature is 37° C. Fermentation reactions have been investigated in some detail by Francis¹⁸ who has found that glucose, maltose and mannose are fermented, the fermentation of glycerol, levulose and dextrin is irregular, and mannitol, galactose, xylose, trehalose, salicin, arabinose, adonite, sucrose, lactose, amygdalin, dulcitol, erythritol, inositol, inulin, raffinose, sorbitol and rhamnose are not fermented. Differential fermentations are of no value. It is killed by exposure to 56° C. for ten minutes. It has been reported that this bacillus contains an endotoxin.

Pathogenicity. Two clinical types of tularemia are recognized, one the glandular or ulceroglandular type, which is the more common, the other the so-called "typhoidal" type. In the first instance the acute stage of the disease is characterized by headache, pains and fever, and a papule appears, fre-

¹⁵ Hesselbrock and Foshay: Jour. Bact., 1945, 49 209.

¹⁶ Tamura and Gibby: Jour. Bact., 1943, 45:361.

¹⁷ Steinhaus and McKee: Pub. Health Repts., 1944, 59.78.

¹⁸ Francis: Jour. Bact., 1942, 43 343.

It will be gathered that the conditions determining the epidemic spread of plague are very stringent. The links in the rat-flea-man chain must be delicately adjusted; a slight fault in one link is sufficient to impair the efficiency of the whole chain, or even to break it altogether. This is doubtless the reason why, in countries where the conditions are not so favourable as in India, plague often has great difficulty in spreading (Robertson 1923). Several times during the present century it has been introduced into England, chiefly at the ports, yet it has uniformly failed to take hold. In East Suffolk, it is true, it was endemic in the rats (Bulstrode 1910-11, Eastwood and Griffith 1914, Macalister and Brooks 1914), having been imported probably in grain; but apart from attacking one or two persons it showed no potentialities for spreading to the human population (see Greenwood 1935).

Plague in other Rodents.

GROUND SQUIRRELS AND OTHER AMERICAN RODENTS.—Plague first appeared at San Francisco in 1900. By 1904 it had spread from the rats to the ground squirrels—*Citellus beecheyi*—and had begun to pass eastward from the coast (McCoy 1910). In 1934 it was found that plague had crossed not only the central valleys of California, but also the Sierra Nevada mountains into the Great Basin section of Oregon. A new species of ground squirrel, *Citellus oregonus*, was shown to be infected. As a result of special field surveys it was learnt that by 1910 no fewer than ten states had been invaded. Three groups of rodents appear to constitute the main primary reservoirs of infection in the west: (1) ground squirrels in the coastal regions and northern part of the inter-mountain plateau; (2) wood rats in the southern deserts; and (3) white-tailed prairie dogs in the plateau regions of Arizona and New Mexico. A more limited reservoir is furnished by chipmunks, mantled ground squirrels, and marmots. Numerous species of fleas, lice, and ticks are concerned in the transfer of infection among these rodents (Eskey and Haas 1940). Cases of human plague have been traced to contact with ground squirrels, but they have been few. During the ten years, 1930-39, only 8 cases were reported (Hampton 1940). If, however, plague should cross the Rocky Mountains and infect the urban rats of the middle west, a more serious situation might arise. In Canada, plague infection seems to be much less widely disseminated; it is confined to ground squirrels and has so far been found only in Alberta and Saskatchewan (Humphreys and Campbell 1947). On the other hand, in South America it is very prevalent, and in the Argentine alone over 70 species of infected rodents have been identified (Hoekenga 1947). The commonest are the guinea-pig (*Microcavia* spp.), the tree rat (*Graomys griseo-flavus*), the vizcacha (a fox-like animal), and the hare (*Lepus europeus*). (For a description of the progress in combating sylvatic plague in the western part of the United States the reader is referred to a series of articles by Meyer in 1936, 1937, 1938 and 1939.)

GERBILLES AND MULTIMAMMATE MICE.—Plague was introduced into South Africa in 1900-2 by rats in forage vessels coming from South American ports. For some years sporadic outbreaks occurred of the usual type in urban areas carried by domestic rodents. About 1912, however, infection began to spread to wild rodents in rural areas. Since then progressive invasion has occurred till now sylvatic plague is established over large areas of the country. The chief wild rodents affected are the gerbilles—the common gerbille (*Tatera brantsii* and *Tatera*

quently on a finger where presumably the bacilli enter the body, which later breaks down and forms an ulcer. The axillary and epitrochlear glands become painful and swollen and may break down with the discharge of purulent material. In cases infected via the conjunctiva, ulcers form on the inner surfaces of the eyelids and the cervical and pre-auricular glands may become tender and somewhat swollen. In the typhoidal type of the disease there are no local symptoms.

During the first week of illness the bacilli may be present in the blood and have been cultured, although in general, cultures made directly from man are not successful and the bacillus is best isolated by guinea pig inoculation and culture from necrotic foci found in liver, spleen and lungs of the pig on autopsy. Direct cultivation from the blood is rarely possible but has been accomplished, and it has been suggested that during the first week of

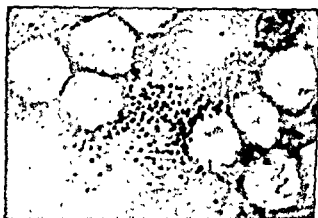


Fig. 98. *Pasteurella tularensis*; coccoïdal form; blood of rabbit (Francis).

the disease an initial bacteremia may occur which, in fulminating cases, develops into septicemia. The bacilli can only rarely be found in smear preparations from human cases. In the experimental disease the bacilli are present in the lymph spaces and phagocytic cells of infected tissues, and their presence within the cells in enormous numbers has caused some workers to suggest an intracellular proliferation of the microorganisms similar to that of the rickettsiae (Chapter 35). Agglutinins are present in the blood in the second week of the disease and may persist in diminishing amounts for at least as long as eighteen years after recovery. The average duration of the disease is two to four weeks. The case fatality is low, 4.8 per cent, and the pathology of the disease in man is not well known.¹⁹

A variety of lower animals have been found to be naturally infected—in addition to the ground squirrels and rabbits noted above, wild rats and mice, woodchucks, opossums, beavers, coyotes, deer, red foxes, ground hogs, muskrats, hogs, skunks, dogs, cats and lambs. The infection also occurs naturally in some birds such as sage hens, grouse and quail. The guinea pig is highly susceptible to artificial inoculation.

Epidemiology. As indicated above, tularemia is acquired by man from

¹⁹ For postmortem findings cf. Mathews: New Orleans Med and Surg Jour, 1938, 90 479.

plague in the winter, when overcrowding in insanitary dwellings is so common. This form of the disease is almost invariably fatal (Jettmar 1923). The incubation period is 3-5 days.

In some countries, such as Manchuria, Transbaikalia, and the Kirghiz Steppes, bubonic plague occurs during the warm weather, and pneumonic plague during the winter. Under these conditions it seems probable that the outbreaks of pneumonic plague arise from cases of bubonic plague complicated by secondary pneumonia.

Bacteriology and Diagnosis of Plague in Man and Animals

Diagnosis of Natural Rat Plague.

During an epizootic the diagnosis of plague in rats can be made almost as well macroscopically as by microscopical examination. The signs and their frequency in *R. rattus* and *R. norvegicus* combined were as follows (Report 1907):

	Per cent
(1) Subcutaneous congestion, particularly in submaxillary region	30.5
(2) Subcutaneous hæmorrhages, particularly in submaxillary region or flank	40.5
(3) Cervical œdema	10.0
(4) Single bubo	73.05
(a) Cervical bubo	75 per cent. of cases of single bubo
(b) Axillary bubo	15.1 " " " " "
(c) Groin bubo	6.1 " " " " "
(d) Sublumbar bubo	3.8 " " " " "
	Per cent.
(5) Multiple buboes	11.67
(6) Granular liver	55.5
(7) Pleural effusion	64.5
(8) Hæmorrhages in lungs and pleuræ	24.0
(9) Hæmorrhages in kidneys and suprarenals	8.5
(10) Granular spleen	4.5

Without doubt the presence of a bubo is the most useful single indication of plague. In the early stage the gland is enlarged, congested, and shows hæmorrhagic points on section. When fully developed, it contains an area of grey necrosis confined to the medulla, or occupying the whole gland. The bubo is hard, and can be moved about under the skin with ease. Microscopically, plague bacilli are found in about 99 per cent. of buboes; in over half the buboes involution forms are present. The bacilli are best demonstrated by staining with carbol-thionin; with this dye they are faintly coloured except at the poles, which stain deeply.

The liver frequently shows a patchy distribution of alternate red and yellow areas—best described as mottling. In many instances there are small, grey or whitish areas of necrosis, giving the organ a stippled appearance as if it had been dusted with grey pepper; as a rule they do not project above the surface. The spleen is sometimes enlarged, and is firm and moulded over the stomach; sometimes it contains granules or actual nodules, which may be discrete or confluent. Occasionally a wedge-shaped portion of the spleen is converted into a cheese-like mass. Pleural effusion is a very characteristic feature; it is clear, abundant,

lower animals either directly or indirectly. The bacilli may enter the unbroken skin of the guinea pig, and possibly this may occur in man through the dressing of infected rabbits and other animals, or the microorganisms may enter by means of minute abrasions on the skin of the hand. Eye infection occurs not infrequently; in fact, such infections were the first human infections with the bacillus observed. Over 90 per cent of the human cases in this country are contracted from rabbits and it is estimated that about 1 per cent of wild rabbits are infected. Jellison and Parker²⁰ have reported that the cottontail rabbit, species of *Sylvilagus* and *S. floridanus* in particular, is by far the most important source of infection in this country, accounting for more than 70 per cent of all human cases in North America. Many wild animals are naturally infected and Burroughs *et al.*²¹



Fig. 99. *Pasteurella tularensis* in hepatic cells of mouse (Francis).

have compiled a list of forty-eight naturally infected vertebrates. Laboratory infection is not uncommon, 56 cases acquired from dissection were reported to 1940. *Past. tularensis* has also been found in streams and is perhaps associated with the epizootics occasionally observed in beavers. Present evidence indicates that fish cannot be infected with *Past. tularensis* and probably play no part in the infection of water.²² Water-borne epidemics have occurred in Russia and Turkey but none has been reported in this country.

The transmission of tularemia by an insect vector is common. In addition to the deer fly, *Chrysops discalis*, *Dermacentor andersoni*, *D. variabilis*, *D. occidentalis*, *Hemaphysalis leporis palustris*, *H. cinnabarina* and *Ixodes ricinus californicus* may carry it. In all probability the wood ticks serve to disseminate the infection in the animal population, and it is of particular interest that the infection is transmitted from the adult tick to the egg and both the larvae and the nymphs are infectious. Tularemia may, then, be in part maintained in the insect population.

²⁰ Jellison and Parker. Amer. Jour. Trop. Med., 1945, 25-349.

²¹ Burroughs *et al.*: Jour. Inf. Dis., 1945, 76-115.

²² Morgau. Amer. Jour. Trop. Med., 1947, 27-399.

The diagnosis of epidemic plague in rats is comparatively simple, but great difficulties are met with in the sporadic form. The lesions are often atypical (Eastwood and Griffith 1914), buboes may be absent, and sometimes nothing characteristic may be noticeable. Williams and Kemmerer (1923), in an examination of rats at Galveston, Texas, found 9 plague-infected rats, none of which showed

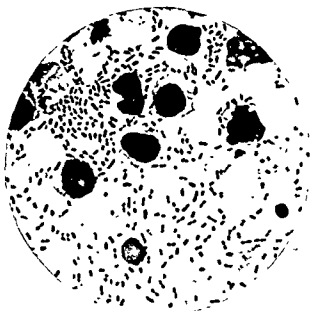


FIG. 292.—*Pasteurella pestis*.

Film from inguinal bubo of infected guinea-pig, showing the bipolar-stained ovoid bacilli ($\times 1000$).

In the search for plague infection among ground squirrels and other similar rodents it has been found more satisfactory to collect the fleas from the animals and inoculate them in pooled batches into guinea-pigs (Eskey and Haas 1910).

When a hitherto uninfected locality is invaded by plague, the rats may succumb rapidly to a septicæmic form of the disease in which buboes are insignificant or absent (Philip and Hirst 1917). Such rats may appear almost normal to the naked eye, though the bacilli are seen microscopically in large numbers in the blood and viscera.

Towards the end of an epizootic, lesions of chronic or resolving plague may be found. The chief of these are: (1) chronic buboes; (2) necrotic areas in the spleen; (3) chronic abscesses in spleen, and more rarely in liver; (4) perisplenitis; (5) scars in spleen; (6) bisected or trisected spleen; (7) adhesions of spleen to surrounding structures. In these lesions bacilli may or may not be found (For a full discussion on the diagnosis of plague in rats the reader is referred to the monographs by Costa 1917 and Pollitzer 1954)

Diagnosis of Plague in Man.

During Life.—A small vesicle is sometimes present on the leg in the early stages of the disease corresponding to the flea-bite. Microscopical examination of the fluid will show the presence of plague bacilli. The bubo, which is generally in the inguinal region, should be aspirated, and the fluid examined microscopically, by culture, and by animal inoculation (Fig. 292).

Plague bacilli are frequently present in the patient's blood, where they may

any macroscopic signs of plague. The infection was discovered by gross, i.e. pooled, inoculations of the tissues of all rats in each day's catch. They advise that the rats should be divided into batches of ten, and the combined spleens or glands of each batch should be emulsified and injected into a guinea-pig. Even this method is not infallible, because at the decline of an epizootic or during an inter-epizootic period the virulence of the bacilli may be so low that the guinea-pig either does not die or dies with atypical lesions; in this case a fresh inoculation should be made from the guinea-pig, and repeated in series till the virulence of the organism is sufficiently exalted to give rise to typical plague.

Tularemia has been found in all parts of the United States, in Japan and in Central Europe,²³ and extensive epidemics have occurred in Russia.

Bacteriological Diagnosis of Tularemia. As indicated above, *Past. tularensis* is difficult to cultivate from infected material. Specimens should be streaked on blood-dextrose-cystine agar. Characteristic minute, drop-like colonies may appear in three to five days but a culture should not be recorded as negative in less than three weeks. The procedure of choice is the intraperitoneal inoculation of a guinea pig with a saline emulsion of the specimen; relatively large amounts are required, usually 4 to 8 ml., of a fairly heavy emulsion. The animal should die in five to ten days. The pathology is characteristic and includes a hemorrhagic edema without pus at the site of inoculation, enlargement of the cervical, axillary and inguinal lymphatics which contain dry caseous material, and small white necrotic areas in the liver and spleen. Smears and cultures may be made but not infrequently the bacilli cannot be found or cultivated. In such instances the diagnosis depends on the guinea pig pathology.

Isolated cultures may be identified by specific agglutination. Conversely, patient's serum may be tested for agglutinins; a titer of 1:80 or higher is usually regarded as diagnostic if there are no *Brucella* agglutinins. In the event that *Brucella* is also agglutinated, the agglutination of *Past. tularensis* occurs more rapidly and to higher titer in tularemia.

Immunity. An attack of tularemia confers a solid immunity, and second infections, when they occur, produce only a local lesion. It may be noted that antibodies (agglutinins) to *Past. tularensis* show some cross-reaction with *Brucella melitensis* and *Brucella abortus*, and for this reason, together with the complex nutritive requirements of these bacilli, some workers have placed them with the *Brucella* group and termed them *Br. tularensis*.

Prophylactic inoculation with vaccines is not successful in experimental animals in that it does not protect against the injection of virulent strains. There is some evidence, however, that vaccine prophylaxis in man confers a useful degree of protection.²⁴ The value of therapeutic antisera is doubtful, its use has been advocated by some²⁵ but others²⁶ regard it as having no significant effect.

²³ Perm. Com. Office Int. Hyg. Pub., Session, May, 1937. Cf. Bull. Hyg., 1937, 12 675

²⁴ Foshay, Hesselbrock, Wittenberg and Rodenberg: Amer. Jour. Pub. Health, 1942, 32 1131

²⁵ Foshay. Medicine, 1940, 19:1.

²⁶ Francis and Felton. Pub. Health Repts., 1942, 57:44.

importance of genetic immunity. For example, in Bombay city, over 2,000 rats were caught each night and examined at the Haffkine Institute. During two years not a single plague-infected rat was found (Sokhey and Chitre 1937). Again, in the region of Paris, where plague was introduced by infected rats in 1917, the resulting epizootic gradually declined after about 1920, so that by 1936 not a single rat out of 3,525 examined was found to be infected (Joltrain 1936). In view of these findings it is difficult to ascribe the increasing immunity of the rats to the persistence of a benign epizootic. It seems more probable that a strain of rats is evolved having a greater degree of natural resistance to plague infection than that of the original susceptible animals.

Prophylaxis, Vaccination, and Serotherapy

Prophylaxis.—The aim of prophylaxis is to weaken or break the rat-flea-man chain by striking at one of its links. The rat constitutes the first link. Complete destruction of all rats and mice is, however, quite impracticable. The animals breed at such a rate as to render futile all attempts at general or permanent extermination. According to the English Plague Commission the progeny of a single pair might number 858 in 16 months (Report 1911). On the other hand, a great deal can be done in localized areas by making use of modern chemical rat poisons such as alphanaphthylurea, sodium fluoroacetate, and Warfarin (3- α -actenolybenzyl-4-hydroxycoumarin), either alone or as supplementary to zinc phosphide, arsenious oxide, and red squill (Report 1946, Macchiavello 1946, Barnett 1948, Hayes and Gaines 1950.) Rats are comparatively harmless so long as they do not come into intimate contact with human beings. The most hopeful policy is to render all houses, granaries, shops, and such places unsuitable as rat habitations. That this can be done successfully has been demonstrated in Java, where between 1914 and 1939 over 1,500,000 houses were remodelled (van Loghem 1939).

The second link in the chain—the flea—is best dealt with by strict personal hygiene and domestic cleanliness aided, when necessary, by the powerful insecticide D.D.T. In the absence of dirt and litter the fleas have nowhere to breed and, even when they have, the judicious application of D.D.T. (dichlorodiphenyltrichloroethane) may rapidly lower the flea infestation of rats by 80 or 90 per cent. Macchiavello (1946), who used D.D.T. in conjunction with sodium fluoroacetate in combating a localized outbreak of plague in Peru, found that 10 per cent. D.D.T. was toxic to the rats, which regularly licked their fur, and that sodium fluoroacetate was toxic to the rat fleas, which died by secondary poisoning from ingesting the blood of the poisoned rats. The possibility that other insects—bugs, flies, ticks—may share in spreading the disease has been carefully considered (Nuttall 1897, 1898, Report 1915c). There seems little doubt that lice and ticks play a part in the transmission of sylvatic plague (Hampton 1940), but their rôle in the spread of plague among rats would appear to be negligible.

Man forms the third link. Four measures are usually advocated: (1) Notification of cases or, in countries where this is difficult, registration of deaths. The diagnosis can be confirmed *post mortem* by splenic puncture. (2) Isolation of cases, which, except in pneumonic plague, is of little value, since man plays but a small part in the spread of the disease. (3) Evacuation, which is sound in theory, since it removes the susceptible population from the infected localities, but is very difficult in practice—at any rate on a large scale. (4) Vaccination, the chief value of which is, or should be, to protect an exposed population while effective

THE HEMOPHILIC BACTERIA

The genus *Hemophilus* as at present constituted is a heterogeneous one. The true hemophilic or hemoglobinophilic bacteria are those whose growth necessitates or is especially favored by the presence of hemoglobin in the culture medium. The inclusion of bacteria other than these nutritionally distinctive types in the genus *Hemophilus* is questioned by many. Bacteria such as the bacillus of whooping cough and the Morax-Axenfeld and Ducrey bacilli are, then, to be associated with the truly hemophilic forms such as Pfeiffer's bacillus in only a tentative way pending a more satisfactory grouping.

THE HEMOPHILIC GROUP

Species	Growth Requirements		Hemolysis
	X Factor	V Factor	
<i>H. influenzae</i>	+	+	—
<i>H. hemolyticus</i>	+	+	+
<i>H. parainfluenzae</i>	—	+	—
<i>H. suis (influenzae suis)</i>	+	+	—
<i>H. canis (hemoglobinophilus)</i>	+	—	—
<i>H. pertussis</i>	—	—	+
<i>H. duplex (Morax Axenfeld)</i>	—	—	—
<i>H. ducreyi</i>	—	—	+

HEMOPHILUS INFLUENZAE (PFEIFFER'S BACILLUS)

Hemophilus influenzae was isolated by Pfeiffer in 1892, and was until relatively recently regarded by many as the etiologic agent of epidemic influenza. Influenza has, however, been shown to be caused by a filterable virus (p. 865), and the name *influenzae* has no etiological significance.

Morphology and Staining. Pfeiffer's bacillus is one of the smallest known pathogenic bacteria, rarely exceeding 1.5μ in length and 0.3μ in

a plague rat at Tjiwidej. A single dose of this vaccine was found to protect 80 per cent. of rats and over 90 per cent. of guinea-pigs against inoculation with a dose of virulent plague bacilli that killed 99-100 per cent. of the control animals. For human beings the vaccine is prepared by washing off with saline a 2-3 day

TABLE 142

HAFKINE'S VACCINE. INCIDENCE AND FATALITY OF PLAGUE IN INOCULATED AND UNINOCULATED PERSONS IN 14 OUTBREAKS. (Modified from Taylor 1933.)

Average Population at Risk.		Inoculated.		Uninoculated.	
Inoculated.	Uninoculated.	Attacks.	Deaths	Attacks.	Deaths
123,134	168,638	803	385	4,014	3,194
Attack rate per 10,000 . .		65		238	
Death-rate per 10,000 . .		31		189	
Case fatality		48 per cent.		80 per cent.	

agar culture grown at 30° C. It can be kept for a month in the ice-chest without loss of potency. At first it was used in two sub-districts of Java, when alternate persons only were inoculated. The results of this controlled trial, in which observations were made over a period of 5 months after vaccination, are summarized in Table 143.

TABLE 143

OTTEN'S VACCINE. MORTALITY FROM PLAGUE IN INOCULATED AND UNINOCULATED PERSONS (Otten 1936).

Sub-district in Java	Inoculated			Uninoculated.		
	Number.	Deaths.	Mortality per 1,000	Number.	Deaths.	Mortality per 1,000.
Bandjaran . . .	18,479	28	1.5	20,669	103	4.9
Batoedjajar . . .	18,956	10	0.5	24,088	110	4.6
Total	37,435	38	1.01	44,757	213	4.75

There were larger numbers of young children in the control than in the vaccinated group; since there is evidence that susceptibility to plague is less in the first six years or so of life than later, the odds were probably weighted somewhat against the vaccinated subjects. Even so the mortality per 1,000 was 4 to 5 times higher in the control group.

Later figures given by Otten (1941) show the fall in the number of deaths in the residency of Priangan. Vaccination of 94 per cent. of the population of two million was carried out in 1935, and continued, including re-vaccination, during 1936-39 (Table 144).

The results are necessarily difficult to interpret, since it is impossible to know what fall in mortality would have occurred in the absence of vaccination. The

thickness. The ends of the cell are rounded, capsules are not generally observed but are present in smooth cultures, spores are not formed, and the bacillus is non-motile. There is a marked tendency to produce threads and other anomalous forms in culture which is, to some degree, a characteristic of strains. Some workers have attempted to differentiate varieties on the basis of morphology, but there is a continuous series of types, ranging from predominantly coccobacillary forms to predominantly longer bacilli and threads, and no sharp distinction can be made. There is a tendency, however, to regard the coccobacillary forms as "typical" and the longer forms as "atypical"; the typical form appears to predominate in strains isolated from pathological processes.

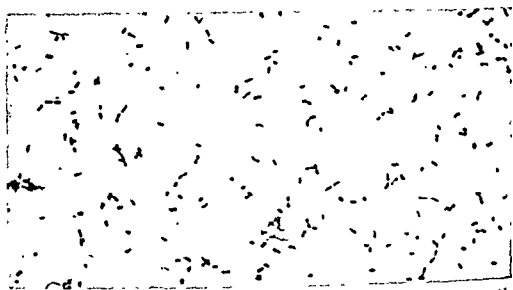


Fig. 100. *Hemophilus influenzae*, pure culture. Note the variability from coccoid to bacillary form and the presence of longer filaments. Fuchsin, $\times 1050$.

On blood agar the colonies of Pfeiffer's bacillus are very small, rounded, discrete and transparent and may reach the size of a small pinhead. If the culture is contaminated with other microorganisms, especially *Staphylococcus aureus*, the colonies are considerably larger, more opaque, and of a grayish white color, and develop most luxuriantly in the neighborhood of the foreign colony, a phenomenon termed the "satellite phenomenon."

These bacilli are somewhat more difficult to stain than most bacteria, Löffler's methylene blue for five minutes or dilute (1:10) carbol fuchsin for ten minutes is satisfactory. They are gram-negative.

Physiology. One of the more fastidious bacteria, Pfeiffer's bacillus requires, as noted above, the presence of blood in the culture medium. It has been found that two substances present in such blood media are necessary to the growth of this bacterium; one, designated as the "X factor," is heat-stable and associated with hemoglobin, and the other, the "V factor," is heat-labile and is found in yeast and various vegetable extracts as well as in whole blood. The satellite phenomenon noted above is due to the formation of the V factor by other bacteria and its diffusion into the medium from the colony. The X factor is replaceable by hemoglobin or hematin.

curative properties in rats. A report on the use of Yersin's, Haffkine's, Lustig's, Terni's, and Brazil's sera was published by the Government of India (Haffkine 1905); none of them affected the fatality of the disease. The English Plague Commission likewise obtained no evidence that serum treatment was beneficial (Report 1912, p. 326). More recently, both Girard (1936) and Pirie and Grasset (1938) found that serum prepared by the inoculation of horses with living avirulent organisms was considerably more potent, as judged by animal tests, than that prepared from dead organisms. In human beings Girard (1941) states that 65 per cent. of patients suffering from bubonic plague recover under serum treatment. Of 70 patients treated with serum by Wagle, Sokhey, Dikshit and Ganapathy (1941), 71.5 per cent. recovered as against only 47.6 per cent. of 82 control patients receiving the routine iodine treatment. The serum was given in doses of 20 ml. intravenously and 20 ml. subcutaneously every day for 2-5 days. Alarming reactions may follow intravenous administration, and it might be better to give more frequent injections intramuscularly.

Chemotherapy.—Schütze (1939b), working with white rats and white mice, found that sulphapyridine had a protective effect similar to that of serum. In the series of patients just referred to, Wagle and his colleagues (1941) treated 53 patients with sulphapyridine and 32 patients with sulphathiazole in 0.5 gm doses every 4 hours up to a maximum of 7 days; the recovery rates were 75.5 and 84.4 per cent. respectively. The results might have been better if a larger dosage had been tried. Neither serum nor sulphonamide is said to be of use in pneumonic plague (Girard 1941, Wagle *et al.* 1941), but Yieh and his colleagues (1948) report the recovery of 3 out of 5 cases treated with sulphadiazine. According to Wayson and McMahon (1944), sulphadiazine is of undoubted therapeutic value in experimentally infected rabbits and guinea-pigs. This compound is now generally considered to be the most active of the sulphonamides. Of the antibiotics, streptomycin is the drug of choice. Herbert (1947) found it to be effective in both experimental bubonic and pneumonic plague in mice and guinea-pigs and to be considerably more active than sulphathiazole. Meyer (1950) advises that, in the treatment of plague, it should be given either alone, or in severe cases combined with chloramphenicol and antiserum, for four days and then replaced by sulphadiazine if progress is satisfactory. Contacts of pneumonic plague should receive 2-3 gm. of sulphadiazine daily for five days.

PASTEURELLOSIS OR HÆMORRHAGIC SEPTICÆMIA OF ANIMALS

The observations of Bollinger and of Kitt in 1878 on an epidemic disease affecting wild hogs, deer, and later cattle in the neighbourhood of Munich constitute the first work of importance on a group of diseases attacking several species of animals, and known collectively as Hæmorrhagic Septicæmia. Kitt was successful in isolating the causative organism and in transmitting the disease to mice and pigeons. A similar organism was obtained by Pasteur in 1880 from fowl cholera, by Loeffler in 1882 from swine plague, by Poels (1886) from septic pleuropneumonia of calves, and by numerous workers during the next few years from diseases in other animals. In 1901 Lignières proposed to unite all these affections into one group under the name of Pasteurellosis, and to call the infecting organisms

Granick and Gilder¹ have shown that the iron protoporphyrin can be replaced with certain other iron porphyrins, but not by porphyrins such as meso-, hemo-, deuto- and coproporphyrins which, however, suppress growth by a competitive inhibition, and such activity is eliminated by methylation of the propionic acid side chains. It seems that these or related compounds are required for respiration—some strains do not require it for anaerobic growth. It has been suggested that hematin is required for the synthesis of catalase, hematin can be replaced by cysteine which would reduce peroxide and make catalase unnecessary. The V factor may be replaced by coenzyme I or coenzyme II but not by nicotinic acid or its amide. Apparently the whole coenzyme molecule must be supplied and it is assumed that the thermolabile substance is of this nature (see p. 118).

A number of media have been devised for the cultivation of the influenza bacillus. Avery's oleate hemoglobin agar, prepared by the addition of sodium oleate and a suspension of erythrocytes to an infusion agar base, is one of the best of these, for not only is the growth of *H. influenzae* enhanced but the growth of streptococci and pneumococci and some other bacteria present in the sputum and nasal mucus is inhibited. The influenza bacillus grows luxuriantly on chocolate agar, prepared by the addition of fresh blood to hot (90° C.) infusion agar, and heavy growths for agglutination and other purposes may be obtained, but this medium does not differentiate and hence is not especially suitable for primary isolation. Fildes' agar, an infusion agar base to which has been added a peptic digest of blood, supports good growth of the influenza bacillus and is used especially by British workers.

No growth occurs on gelatin or potato. Milk, containing blood, is rendered slightly alkaline by some strains. Nitrate is reduced to nitrite. Some strains—about 50 per cent—form indol. Fermentation reactions are variable, some strains being inactive while others ferment dextrose and other carbohydrates. Some strains are hemolytic while others are not. The hemolytic strains of *H. influenzae* do not appear to be clearly marked from the non-hemolytic strains by any other differential characters, since indol production and carbohydrate fermentation occur in both groups.

The influenza bacillus shows little resistance toward external conditions. Desiccation is quickly fatal. A pure culture suspended in water and then dried on silk threads loses its vitality within twenty-four hours, in dried sputum life is maintained somewhat longer, but not, as a rule, beyond forty-eight hours. The bacilli are readily killed by disinfectants. Even under favorable conditions artificial cultures soon die out, and in order to preserve vitality subcultures must be made every four or five days, chocolate agar is suitable for maintaining stock cultures.

Varieties. Subdivisions of *H. influenzae* have been made by a number of workers, but whether such varieties deserve the dignity of species standing accorded some of them by the Bergey (1948) classification is open to serious question. The morphologically "typical" and "atypical" forms have been noted above, but these are not regarded as separate species. Other distinctions which have been made on the basis of hemolysis and nutritive requirements may be summarized briefly:

¹ Granick and Gilder Jour. Gen. Physiol., 1946, 30 1.

(1921, 1922a, b, 1923), working with rabbits suffering from snuffles—a chronic form of the disease—observed the organism in the nose of a large proportion of normal animals. Webster (1924a, b) found that preceding an outbreak there was a rise in the normal carrier rate. The susceptibility of rabbits varied greatly, some being highly resistant, others succumbing with ease. Similar variation in the susceptibility of fowls to natural and experimental infection was likewise noted by Rice (1926). These observations suggest that natural infection occurs through the nose, and that it is dependent on the presence of carriers.

Pasteurella Infections in Man.—Though several of the early authors described diseases in man that they considered to be due to *Pasteurella* infection, few of the accounts carry conviction. Reviewing the literature, Regamey (1939) (see also Lévy-Bruhl 1938) traced ten cases that were probably, and six cases which he thinks were certainly, due to this organism. Of the six genuine cases, three were characterized by pleurisy, two by meningitis, and one by a local lesion following the scratch of a cat. Of more recent years several genuine cases have been recognized, some of them recorded (Foerster 1938, Mulder 1938, Boisvert and Fousek 1941, Ludlam 1944), and some of them not. Allott and his colleagues (1944) reported six cases, three following cat bites and three following dog bites. Three of the cases were complicated by osteomyelitis. Localized disease is commonly followed by recovery, but generalized cases have usually proved fatal. Infection may occur without accompanying illness; in our own experience, for example, an animal house attendant who carried *Pasteurella septica* in his nose for several months remained quite well.

Protection, Vaccination, Serotherapy, and Chemotherapy.

It was when working with chicken cholera that Pasteur observed a spontaneous diminution of virulence in a culture which he had left for some weeks at room temperature in contact with the air. This culture, on inoculation into fowls, proved harmless. When these fowls were re-inoculated shortly afterwards with a virulent culture, instead of dying of hæmorrhagic septicæmia they recovered rapidly after a brief indisposition.

Vaccines for the prophylaxis of pasteurellosis are made chiefly from strains attenuated by heat or chemical agents. In some instances their use appears to have yielded satisfactory results (Brimhall and Wilson 1900, Chamberland and Jouan 1906, Hutyrá and Marek 1912, Magnusson 1914). The work of Priestley (1936) seems to show the importance of using a vaccine prepared from virulent capsulated bacilli. To avoid destroying the antigenic properties of the capsule the organisms should be killed by heating at 56° C. for not more than 30 minutes. In view of the existence of four serological types, Roberts (1947) advises that vaccines should be polyvalent. Immune sera prepared by injection of horses with living organisms or with body exudates are sometimes employed therapeutically (Schrop 1908). The most promising method appears to be combined active and passive immunization.

According to Cardoso, Reis and Nobuga (1939), sulphanilamide has a protective effect in chickens experimentally inoculated with *Pasteurella*.

Other Diseases Associated with *Pasteurella* or *Pasteurella*-like Organisms.

Pasteurella is a common secondary invader in many diseases of animals. It has been found especially in swine fever, in which it gives rise to severe pulmonary

(1) The hemolytic and non-hemolytic varieties. The non-hemolytic form is designated *H. influenzae* by Bergey and the hemolytic form *H. hemolyticus*. Both require the X and V nutritive factors. These are generally regarded as a single species, *H. influenzae*.

(2) The swine influenza bacillus, *H. influenzae suis* or *H. suis*, which closely resembles Pfeiffer's bacillus except that it is relatively inert biochemically and differs immunologically. This bacterium, in association with a filterable virus, is causally associated with swine influenza. Both X and V factors are required for growth.

(3) The para-influenza bacilli, *H. parainfluenzae*, which closely resemble *H. influenzae* except that only the V factor is required for growth and these bacteria may be cultivated on agar containing serum or ascitic fluid. Although these bacilli are defined as non-hemolytic, hemolytic strains showing the same nutritive requirements are found.

(4) *H. canis* (*H. hemoglobinophilus*, *H. hemoglobinophilus canis*), found in the preputial secretions of dogs. It closely resembles *H. influenzae* except that it requires only the X factor for growth.

Variation and Antigenic Structure. As tested by direct agglutination, *H. influenzae* is antigenically heterogeneous. Pittman,² however, has described rough and smooth forms in which the smooth form is encapsulated. By means of the agglutination test the encapsulated bacilli are found to fall into five immunological types, designated A, B, C, D, E and F, and the antigens responsible are specific polysaccharides.³ Diagnostic and therapeutic antisera may be prepared for each of these types.⁴ It appears that many strains isolated are in the rough form, and these, by the agglutination test, are immunologically heterogeneous. Antigenic proteins may be extracted from the cell substance of influenza bacilli.⁵ One of these, fraction M, was found to be common to most strains of *H. influenzae*, indicating an immunological homogeneity demonstrable by precipitin tests with appropriately prepared antigens. It will be clear that the antigenic structure of *H. influenzae* is by no means fully understood as yet. The influenza bacilli appears to be immunologically related to certain of the pneumococcus types, Type A shows cross-reactions with pneumococcus Type 6b and Type B cross reacts with pneumococcus Type 6 and Type 29. The immunological relationship of other *Hemophilus* species to the influenza bacillus and to one another is not yet known.

The S-R dissociation noted by Pittman is to some degree reversible by growth in the presence of anti-R immune serum. The relation of virulence to this dissociative change is not known.

Toxins. As in the case of many other bacteria, the cell substance of the influenza bacillus is toxic to experimental animals, mice in particular, upon parenteral inoculation. Toxic substances are produced in fluid cultures, are filterable, and may appear in appreciable quantities after six to eight hours'

² Pittman Jour. Exp. Med., 1931, 53:471.

³ For the preparation of these see MacPherson, Heidelberger and Alexander: Jour. Immunol., 1946, 52:207.

⁴ See Alexander, Leidy and MacPherson: Jour. Immunol., 1946, 54:207.

⁵ Platt: Australian Jour. Exp. Biol. Med., 1939, 17:19.

According to Cook (1952) they can be demonstrated most readily by making use of their slightly acid-fast property. Smears are stained with a 1/10 dilution of 1 per cent. carbol-fuchsin for 30 seconds, washed, and counterstained for 10-15 seconds with Loeffler's methylene blue. The cytoplasm of cells and cellular debris are stained blue or purplish blue; the cell nuclei and the pseudotubercle bacilli are stained red.

Pseudotuberculosis of Mice.—This disease is due to a short Gram-positive diphtheroid bacillus, *C. murium*, described independently by Kutscher (1894) and Bongert (1901) (see Chapter 17).

Pseudotuberculosis of Sheep.—This disease has been reported in Europe, America, and Australia. It is caused by a short, non-motile, Gram-positive diphtheroid organism, known as the Preisz-Nocard bacillus or *C. ovis* (Preis 1894). The same organism appears to be responsible for ulcerative lymphangitis of horses and perhaps for contagious acne of horses (Hutyra and Marek 1912) (see Chapter 17).

Pseudotuberculosis of Pigs.—Both Chaussé (1916) and Velu (1922) describe a disease of pigs under the name of pseudotuberculosis. The peri-pharyngeal lymph nodes appear to be chiefly affected, and the caseous lesions produced resemble those of true tuberculosis very closely. Visceral lesions may or may not be present. The cause of the disease is unknown.

Pseudotuberculosis of Man.—Topping, Watts and Lillie (1938) recorded what they considered to be the fifth authentic case in medical literature. Hussig, Karrer and Pusterla (1919) reviewed 17 cases and described 2 of their own. The usual clinical picture is that of a typhoid-like disease with swelling of the liver and spleen and an almost uniformly fatal outcome. Jaundice increasing in intensity towards the end is not uncommon. Death occurs in 2 to 3 weeks. At post-mortem the liver is studded with nodular necrotic foci or abscesses, 1-20 mm. in diameter, and the spleen shows a diffuse lymphoid hyperplasia. *Past. pseudotuberculosis* can be cultivated from the liver nodules after death and sometimes from the blood during life. The disease appears to affect mainly adult males.

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incubation. Relatively large quantities of the filtrate (2 to 4 ml.), however, are necessary to produce death in rabbits, and it is probable that a true exotoxin is not formed.

Pathogenicity for Man. The pathogenicity of Pfeiffer's bacillus for man is shown by the occurrence of cases of meningitis, mostly in infants and of a high fatality, in which *H. influenzae* is found in pure culture in the cerebro-spinal fluid. These cases of "influenzal meningitis," while not very numerous, show that certain strains of this microorganism possess a definite invasive power. *H. influenzae* meningitis is the fourth commonest form of purulent meningitis, it occurs most often in the second six months of life and the case fatality rate is between 90 and 100 per cent. Occasional cases of otitis media, appendicitis, sinusitis and other localized infections may be caused by this bacterium.

In infections of the respiratory tract the influenza bacillus is frequently present, and it is found on autopsy in pneumonic lesions under conditions where its destructive action upon the tissues can hardly be doubted. Whether it is present as a primary or secondary invader in these cases is more uncertain. Its common occurrence in diseases like measles, whooping cough and tuberculosis indicates that its growth on human tissue is favored by the presence of other infecting agents. It seems probable that in respiratory infections *H. influenzae* commonly follows in the wake of some other microorganism.

It may be noted that the relation of this bacillus to influenza is very likely that of a secondary invader to the initial virus infection. It is frequently but not invariably present in cases of influenza and, of course, occurs in the absence of this disease. In the case of swine influenza, however, the influenza bacillus is, in association with a filterable virus, causally related to the disease (p. 869).

Pathogenicity for Lower Animals. Except in swine influenza, the influenza bacillus is probably not a natural pathogen of lower animals. Upon intraperitoneal inoculation into laboratory animals, mice, guinea pigs and rabbits, large doses of these bacteria produce death within one or two days. Whether this is an actual invasion rather than a toxemia is uncertain; the bacilli may be found in the peritoneal exudate but usually not in the heart's blood, and petechial hemorrhages may be observed scattered over the peritoneum and, sometimes, the pleura. Certain strains produce a fatal infection in mice on intracerebral inoculation. As tested by intraperitoneal inoculation, the virulence of *H. influenzae* varies greatly from strain to strain; the virulent strains are in a minority and strains from influenzal meningitis are generally among the more virulent.

By inoculation of the mucous membrane of the upper respiratory tract of normal monkeys Blake and Cecil⁶ succeeded in producing an acute upper respiratory disease resembling influenza with pathologic changes similar to spontaneous, uncomplicated *H. influenzae* pneumonia in man. Inoculation experiments upon man with pure cultures of these bacilli, however, have given a surprisingly large number of negative results.⁷

Other hemophilic bacteria have been described in connection with diseases

⁶ Blake and Cecil. Jour. Exp. Med., 1920, 32 691, 719.

⁷ Cf. Rosenau. Jour. Amer. Med. Assn., 1919, 73 311. McCoy and Richey. Pub. Health Reps., 1919, 34 33.

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of lower animals. *H. canis*, for example, has been found in association with inflammation of the prepuce in dogs but is apparently harmless. Other hemophilic bacilli such as *H. bovis*, *H. gallinarum*, *H. muris*, *H. ovis* have been isolated from lower animals. Their relationship to *H. influenzae* and other better known species is not clear.

THE KOCH-WEEKS BACILLUS

A small bacillus, first observed by Koch in 1883 in a series of eye inflammations in Egypt, was successfully cultivated by Weeks in New York in 1887, and is now recognized as the cause of a world-wide and highly contagious form of conjunctivitis sometimes known as pink-eye.

It has been stated that the Koch-Weeks bacillus will grow on serum agar, or a mixture of glycerol agar and ascitic fluid or, at times, even on nutrient

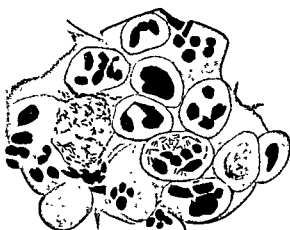


Fig 101. Koch-Weeks bacillus in conjunctivitis, $\times 900$ (Axenfeld, Kolle and Wassermann).

agar. Others have found, however, that both the X and V factors required by *H. influenzae* are required by the Koch-Weeks bacillus. It is, in fact, highly probable that this bacillus is identical with *H. influenzae*.

The bacilli possess slight powers of resistance, and it is unlikely that dust is a means of conveying the infection. Direct contact with infective material through the medium of hands, towels, handkerchiefs, etc., is the usual mode of transmission. A peculiar form of hand infection due to an organism probably identical with the Koch-Weeks bacillus has been described.

HEMOPHILUS PERTUSSIS

Bacilli resembling *H. influenzae* were reported by early observers as occurring in a large proportion of cases of whooping cough. Although there are minor differences in the descriptions of these organisms as given by different observers, the cultural and morphological characters are essentially similar, and there seems little doubt that Spengler, Jochmann and Kraus, Wollstein, and Davis discovered the same bacillus. More definite results were obtained by Bordet and Gengou⁸ who found in the bronchial exudate from cases of whooping cough a characteristic short oval bacillus which grew

⁸ Bordet and Gengou Ann. Inst. Pasteur, 1906, 20 731.

have been successful in this end (see Shibley *et al.* 1929, Dochez *et al.* 1930, 1931*a, b*, 1932, 1933, 1936, Long *et al.* 1931, Andrewes 1949, 1950); and there is now no reason to doubt the essential correctness of the conclusion advanced by Foster (1917) that the common cold is a virus disease.

Little is known about the virus itself. This is mainly because cultivation has so far proved unsatisfactory and because, apart from the chimpanzee (Dochez *et al.* 1930), man is the only susceptible subject on which pathogenicity experiments can be made.

Several apparently successful attempts to grow the virus in tissue culture (Dochez *et al.* 1931*a, b*, 1936, Powell and Clowes 1931) or in the developing chick embryo (Topping and Atlas 1947, Pollard and Caplovitz 1948, Ward and Proctor 1950) are on record, but the fact that these cannot be uniformly confirmed by competent workers shows that the correct conditions for cultivation are as yet not perfectly understood (see Andrewes 1950).

Hopeful results, however, were obtained by Andrewes and his colleagues (1953), who grew the virus in tissue cultures of embryonic human lung. They propagated it through 10 serial cultures and succeeded in producing colds in a certain proportion of human volunteers with the last passage strain.

The size of the virus is in doubt; it may be 40-50 $m\mu$ in diameter or it may perhaps be even smaller—20 $m\mu$ or less. In nasal filtrates it survives at least 2 years at -76°C. , a month at -10°C. , and 3 days at 4°C. (Andrewes 1949).

There is reason to believe that different strains of virus differ in virulence and spreading power (Andrewes *et al.* 1951); but as all investigations have to be made on human volunteers, 40 per cent. of whom in England and Wales are temporarily immune to colds, exact information is difficult to obtain.

Though bacteria may play a part in determining complications of the common cold, there is little reason to believe that they are responsible for the primary illness. Observations have failed to reveal any consistent change in the bacterial flora of the nasopharynx accompanying the onset of an acute cold.

In an investigation undertaken in Manchester during the period 1926-28 (see Report 1930) a nasopharyngeal swab was taken each month from 100 persons who volunteered to assist in the inquiry. Additional swabbings were taken from these persons whenever they contracted a cold. Information was thus available for a considerable number of

TABLE 146

	With Colds (110 Swabs).		Without Colds (1,731 Swabs).	
	Number +.	Per cent. +	Number +.	Per cent. +.
<i>H. influenzae</i>	52	47.27	984	56.85
Hæmolytic streptococci .	19	17.27	229	13.23
Pneumococci	30	27.27	446	25.77

swabbings, taken from the same persons at times when they were suffering from a cold, and at other times when they were not. The figures referring to the frequency of *H. influenzae*, hæmolytic streptococci and the pneumococcus, are set out in Table 146. They refer only to those persons who contracted a cold during the period of observation; and they do not include the results obtained in cases which, on clinical grounds, were diagnosed as influenza.

Pneumococci and hæmolytic streptococci were slightly more frequent in those that had colds than in those that had not, but the difference was slight and insignificant; *H. influenzae* was more frequently isolated at times when no sign of a cold was present. It

feebly on a special medium they devised. Earlier named *Bacillus pertussis*, this microorganism is now known either by that name or as *Hemophilus pertussis*, or, more casually, as the Bordet-Gengou bacillus.

Morphology and Staining. The Bordet-Gengou bacillus is a small ovoid rod from 1.0 to 1.5 μ in length and 0.3 to 0.5 μ in breadth. The majority of the bacteria occur singly, although they may occasionally be seen in pairs end to end, chains do not occur in smears of bronchial exudate, but short chains may be seen in cultures in liquid media. This morphology is relatively constant and there is not the tendency to the formation of thread like and other aberrant forms which is exhibited by the influenza bacillus. *H. pertussis* is non-motile and non-spore-forming, and the smooth form is encapsulated.



Fig 102. *Hemophilus pertussis*, pure culture. Fuchsin, $\times 1050$.

On the Bordet Gengou medium the colonies are smooth, raised and glistening, with a metallic or pearl-like luster, and are larger and more opaque than those of the "whooping cough" bacillus. The colonies are not required for the purpose of the culture, and the growth is sticky and tenacious. On blood agar the colonies acquire a slight hemolysis.

by the culture, and the growth is sticky and tenacious. On blood agar the colonies are surrounded by a narrow zone of hazy hemolysis.

The bacilli stain with a little difficulty and, as in the case of the influenza bacillus, methylene blue or dilute carbol fuchsin must be applied for five to ten minutes, carbol toluidine blue is recommended by some workers, and stains the bacilli a lilac color. A tendency to bipolar staining may be observed. They are gram negative.

Physiology. *H. pertussis* is difficult to cultivate upon primary isolation, and the Bordet Gengou medium upon which it grows readily consists of 1 per cent glycerol agar or glycerol broth made with macerated potato and added to an equal volume of human or rabbit blood. By repeated passage on media containing less and less blood, however, it may be acclimatized and will, in time, grow, although sparsely, upon ordinary nutrient agar. It does not, therefore, require the V and X factors that are essential to the development of

survival from one epidemic to another, its extraordinary variability and its incalculable behaviour—a series of problems that baffle the most earnest students of the disease.

The clinical syndrome that justifies a diagnosis of influenza is singularly difficult to define (see Stuart-Harris *et al.* 1938), and partly for this reason the history of the disease through the centuries can be traced only with great caution. The pandemic of 1890 was followed by intermittent epidemics, possibly of increasing severity, which culminated in the great pandemic of 1918–19. This was one of the worst plagues of human history. It was worldwide in its distribution; it is believed to have killed between ten and twenty million persons; and it destroyed more lives in a few months than the Great War did in four years (see Report 1920). Since then influenza has occurred at fairly regular intervals, but never with anything like the same severity. In different outbreaks the attack rate varies from 1 to 30 per cent., the case-fatality rate is usually low, and the deaths, which occur mainly in the very young and the very old, are often attributable to a complicating pneumonia (see Stuart-Harris *et al.* 1949). The fact, however, that when it strikes isolated communities, such as those of the Eskimos, it may have an attack rate of 100 per cent. and a case-fatality rate of 20 per cent. (Nagler *et al.* 1949; see also Isaacs *et al.* 1950), suggests that under ordinary civilized conditions the population possesses some degree of immunity to the infection. This is borne out by the observation that during an epidemic quite a considerable proportion of those exposed show a rise in the antibody content of their blood in the absence of any clinical manifestations of disease (see Report 1948).

Most outbreaks in the northern hemisphere occur during the winter or early spring months. Those due to Type A virus tend to recur every 2 or 3 years and to be epidemic: those due to Type B virus recur every 3 to 6 years, and are usually milder and less widespread (see Report 1946). Mixed outbreaks are sometimes observed. Since 1917 there has been a change in the type of the virus, and variants are now being met with which are often different in each successive outbreak. This makes the duration of immunity after an attack very difficult to determine; but there is some evidence to show that to the homologous virus it may last for as long as 4 years (Pickles *et al.* 1947).

How the virus survives from one epidemic to another, and what it is that determines an epidemic outbreak, cannot be said. Though during, or occasionally just before, an outbreak the virus may be isolated from healthy persons (Taylor 1949), the existence of the carrier state between epidemics has not yet been satisfactorily proved. It is believed by some workers that the disease is maintained by sporadic cases, and that when a sufficiently large susceptible population has accumulated, the infection takes on a rapid spread (see Burnet 1951). How far this is due to the appearance of a new variant to which the population possesses little immunity (see Smith *et al.* 1951), and how far to a strain to which immunity is waning, still remains to be determined. Again, whether the infection arises in one country and spreads in sequence to others, or whether the epidemic has a multicentric origin is doubtful; there is some reason to think that either is possible (see Andrewes 1950).

The *Ætiology* of Influenza

In view of our present knowledge the long controversy on the causative rôle of *H. influenza* (Pfeiffer 1892) has lost all but historical interest.

the hemophilic bacteria. The optimum temperature is 37° C., and the bacillus is aerobic and facultatively anaerobic.

H. pertussis is biochemically inactive. It does not form indol, does not reduce nitrates, and does not ferment any sugars. It is in part because of this inactivity and lack of the strict nutritive requirements that characterize the hemophilic bacteria that many prefer not to include it in the genus *Hemophilus*.

The resistance of *H. pertussis* to deleterious influences is feeble and of the same order as that of the influenza bacillus. It is killed by exposure to 55° C. for thirty minutes.

Toxins. As in the case of Pfeiffer's bacillus, the cell substance of *H. pertussis* is toxic upon parenteral injection into experimental animals and to about the same degree. Recent work seems to indicate definitely that an endotoxin is present in these bacilli which may be separated as a watery extract of disintegrated cells. There appear to be two fractions, one heat-stable and the other heat-labile and inactivated in thirty minutes at 56° C.⁹ On intravenous inoculation in rabbits the heat-stable fraction produces a hyperglycemia and the heat-labile fraction a hypoglycemia. Intratracheal inoculation in rabbits produces an edematous reaction followed by a lymphocytic infiltration about the blood vessels and bronchi which is reported to be similar to the changes produced in the lung in whooping cough. Extracellular toxin found in cultures of *H. pertussis* appears to be liberated endotoxin.

Variation and Antigenic Structure. Unlike the influenza bacillus, *H. pertussis* is generally in the smooth state when isolated from the body on an optimal medium, and is immunologically homogeneous. Leslie and Gardner¹⁰ found that their newly isolated strains fell into four immunologic groups which they designated as Phases I, II, III and IV. Although not obviously rough, Phases III and IV are somewhat rougher in appearance and less stable in saline suspensions than the bacilli of Phase I. The S-R dissociation of *B. pertussis* occurs readily on culture on artificial media, even on blood agar, and it seems probable that these phases do not represent distinct immunological types but rather successive stages in the S-R transformation. The importance of the existence of the bacilli in Phase I for the preparation of vaccines and the like has been stressed by many workers in the last few years; it will be clear, however, that in all probability this is but another way of saying that the bacilli must be in the smooth state.

The S-R change, of which the above phases represent the early stages, proceeds to the obviously rough stage with consequent alterations in colonial morphology and loss of virulence.

As indicated elsewhere (p. 501), *H. Brucella bronchiseptica*. Eldering and bacilli isolated from a small proportion of cases of whooping cough which designate *Bacillus para-pertussis*. These bacilli differ from *H. pertussis* in

⁹ Evans and Maitland: Jour. Path. Bact., 1937, 45:715; Erich, Bondi, Mudd and Flosdorf: Amer. Jour. Med Sci., 1942, 204:530; Evans: Jour. Path. Bact., 1943, 55:269. Sprunt and Martin: Amer. Jour. Path., 1943, 19:255.

¹⁰ Leslie and Gardner: Jour. Hyg., 1931, 31:423.

¹¹ Eldering and Kendrick: Jour. Bact., 1938, 35:561.

In 1946 a modified Type A strain appeared in Australia, generally referred to as A-prime (see Salk and Suriano 1949), which displaced throughout the world the more classical Type A strains. Modifications of the A-prime type were subsequently recognized; and a further type, to which the provisional denomination Type C was given, was described by Taylor (1949) and Minusc, Quilligan and Francis (1951). Numerous variant strains have been found in different epidemics, and from even localized outbreaks strains presenting certain biological differences have been isolated (Kalter *et al.* 1948, Sigel 1949, Smith *et al.* 1951, Isaacs *et al.* 1952).

Properties of the Virus.—Various estimates have been made of the size of the influenza virus.

Elford, Andrewes and Tang (1936), using the gradocol membrane technique, found it to fall between the limits of 80 and 120 $m\mu$; and Elford and Andrewes (1936), using the

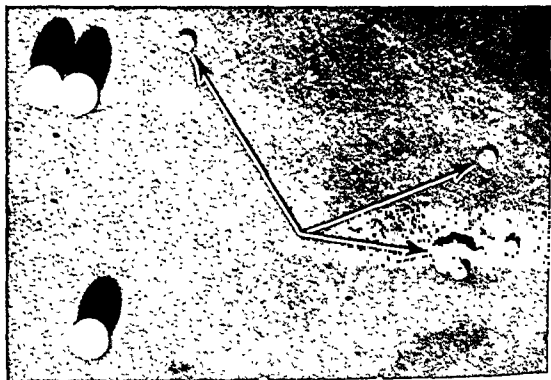


FIG. 292a. Electronmicrograph of influenza virus particles (indicated by arrows) and polystyrene latex particles used for comparison. (Gold manganin shadowed. $\times 25,000$.) (Kindly supplied by Dr. A. Isaacs.)

centrifugation technique, estimated it at between 87 and 99 $m\mu$. Other estimates made by the centrifugal method place it at 77.6 $m\mu$ (Taylor *et al.* 1943), and 80 $m\mu$ (Lauffer and Stanley 1944), and by electronmicrography at 77.6 $m\mu$ (Sharp *et al.* 1944) and 115 $m\mu$ (Lauffer and Stanley 1944). The B virus appears to be slightly larger than the A virus; Chu, Dawson and Elford (1949) quoted figures of 103 and 90 $m\mu$ respectively. The same observers recorded that in the amniotic or allantoic fluid both types may form not only spherical but also filamentous forms (see also Wyckoff 1951). According to Taylor and his colleagues (1944) the swine influenza virus resembles in size Virus A.

The virus was grown in tissue culture by Francis and Magill (1935b) and on the chorio-allantoic membrane of the developing chick embryo by Smith (1935) and Burnet (1935b). From human material it is best isolated by injection into the amniotic cavity; it can then be adapted to grow in the allantoic cavity. Multiplication also occurs in the yolk-sac, but a larger inoculum is required than

that they grow readily on ordinary nutrient agar upon isolation and produce an alkalinity in litmus milk in two to four days. Immunologically, they are related to both *H. pertussis* and *Br. bronchiseptica*.

Pathogenicity for Man. Interest in *H. pertussis* has centered chiefly about the possible etiological role of this microorganism in whooping cough. Although this bacillus was early observed in the bronchial exudate, where it is present in enormous numbers in the early stages of the disease, failure to reproduce the disease in laboratory animals and other considerations led many to question the etiological significance of *H. pertussis*. For example, the presence of inclusion bodies (p. 842) observed by McCordock¹² has suggested a filterable virus etiology. At the present time, however, the chain of evidence formalized as Koch's postulates appears to be complete, and it may reasonably be concluded that *H. pertussis* is the causal agent of whooping cough.

The microorganism is constantly present in the disease, most frequently in the catarrhal stage which is known to be the most contagious, less frequently in the paroxysmal stage, and disappears during the decline, being rarely found after the fourth week of disease. It is not found in healthy persons who have not been in contact with whooping-cough patients, or in patients with infectious diseases other than whooping cough. By the cough-convulsion test Chievitz and Meyer¹³ *H. pertussis* has been demonstrated in 98 per cent of whooping cough examined by Danish workers and in 98 per cent by Sauer and Hambrecht¹⁴ in this country. The cellular infiltration and necrosis of the bronchi seen at autopsy in cases of whooping cough have been reproduced in the chick embryo by Gallavan and Goodpasture,¹⁵ an interstitial pneumonia with leucocytic infiltration about the vessels and bronchioles and mucous secretion on the bronchial epithelium has been produced by intratracheal inoculation in the mouse¹⁶ and in the rat by intranasal inoculation.¹⁷ Rich¹⁸ has produced symptoms similar to those of whooping cough in man by the inoculation of chimpanzees, and MacDonald and MacDonald¹⁹ have recorded experimental pertussis in man.

Significant work upon the relation of *H. pertussis* to the characteristic manifestations of whooping cough has been carried out by Mallory and his co-workers.²⁰ The production of a mild toxin by the bacillus and the absorption of the toxin seem to be shown by the exudation of leucocytes into the lumen of the trachea and bronchi, by slight changes in the lymph nodules of the spleen, lymph nodes and gastro-intestinal tract, by the occurrence of the well-known lymphocytosis of whooping cough, and by the production of the antibody which makes possible the specific complement-fixation reaction. Possibly the cilia are damaged by a toxin, but this is not certain. More important than

¹² McCordock: Proc. Soc. Exp. Biol. Med., 1932, 29 1288.

¹³ Chievitz and Meyer: Ann. Inst. Pasteur, 1916, 30 503.

¹⁴ Sauer and Hambrecht: Jour. Amer. Med. Assn., 1930, 95-263

¹⁵ Gallavan and Goodpasture: Amer. Jour. Path., 1937, 13 927.

¹⁶ Burnet and Timmins: Brit. Jour. Exp. Path., 1937, 18 83, Bradford: Amer. Jour. Path., 1938, 14 377.

¹⁷ Hornibrook and Ashburn: Pub. Health Repts., 1939, 54 439.

¹⁸ Rich: Bull. Johns Hopkins Hosp., 1932, 51-346, Rich *et al.*: Science, 1932, 76 330.

¹⁹ MacDonald and MacDonald: Jour. Inf. Dis., 1933, 53-328

²⁰ Mallory *et al.*: Jour. Med. Res., 1912-13, 22-115, 391.

Another variation, seen in some Type A strains, results from continued passage on the chorio-allantois. A highly virulent endotheliotropic variant may be produced that kills the embryo with gross hæmorrhagic lesions of the brain in 48 hours or less (Burnet 1945). The production of variant strains by the process of *recombination*, occurring as the result of growing together two strains with different properties (Burnet and Lind 1951), was described in Chapter 41.

In the study of variation considerable use has been made of the response of the viruses to the inhibitory agents present in normal and immune sera. Rabbit serum contains two agents capable of preventing hæmagglutination. One is the Francis factor already referred to, called α by Smith, Westwood and Belyavin (1951): it is sensitive to R.D.E. and inhibits heated virus more strongly than living virus. The other, or β factor, is insensitive to R.D.E. and inhibits living more strongly than heated virus. A lecithin-like factor present in the normal serum of man and various animals and capable of inactivating the virus without affecting its hæmagglutinating power was described by Utz (1949). The inactivating or neutralizing power of serum from immune animals seems to be independent of its action on hæmagglutination (Walker and Horsfall 1950).

Growth Cycle.—The mode of multiplication of the influenza virus has already been discussed in Chapter 41, and there is no need to do more here than to remind the reader that, after inoculation into the allantoic sac, the virus disappears and cannot be demonstrated again in its fully developed form for 5–6 hours, though an antigenic component of the virus is detectable by complement fixation after 2–4 hours (Hoyle 1950). Whether this indicates that the fully formed virus results from the aggregation of smaller units that have multiplied separately, or whether another explanation is more probable is still a matter for dispute (see Cairns *et al.* 1952, Fazekas de St. Groth and Graham 1954).

Antigenic Structure.—Antigenically the A and B viruses are distinct. Within each type there is considerable heterogeneity. Variants of the A virus were described among others by Magill and Francis (1936), Magill and Sugg (1944), Friedewald (1944), Hilleman, Mason and Rogers (1950), Isaacs and Andrewes (1951), and Hirst (1952), and variants of the B virus by Eaton and Beck (1941) and Gordon (1942). Some of the Type A strains are related to the swine influenza virus (Glover and Andrewes 1941, Hudson *et al.* 1943). Studies have revealed the existence of at least two antigenic fractions in the influenza virus: (a) a fraction intimately associated with the elementary virus particle, readily adsorbed by fowl red blood corpuscles, and showing a strain specificity in the complement-fixation test, and (b) a soluble fraction which remains in the supernatant fluid of centrifuged specimens, which is not adsorbed by red cells, and which shows a group specificity in the complement-fixation test (see Fulton and Dumbell 1949, Hoyle 1952). The hæmagglutinin can be extracted by ether from the virus particles. Apparently it consists of a protein portion carrying the enzymic activity and a combining group having an affinity for the red cells. From the ethereal extract a serologically active lipid can be separated with properties suggesting that it is derived from the host cell (Hoyle 1952). In the unheated state the A and B viruses show no antigenic affinity, but if they are heated to 60° C., cross-fixation occurs between them. The cross-reactions are thought to be due chiefly to an antigen derived from the protein of the host cells (Smith 1952).

Resistance.—According to Knight and Stanley (1944), the purified virus is inactivated promptly by 5 per cent. phenol, in a week by 0.5 per cent. phenol, and in a month by 0.1 per cent. phenol. The effect of other disinfectants was studied by Dunham and MacNeal (1944). According to Chu (1948), formalin in low concentration inactivates influenza Type B virus without appreciably impairing its hæmagglutinating function. A differential destruction of the various properties of the influenza virus may likewise be brought about by ultraviolet irradiation (Henle and Henle 1947).

Animal Pathogenicity.—The influenza virus is pathogenic for the ferret, the mouse and the hamster on intranasal injection. The mouse is fairly resistant to virus present in the nas

to tion

toxic action seems to be the mechanical disturbance caused by the presence of the bacilli in the respiratory tract. By their presence in enormous numbers, dozens to a hundred or more between the cilia of a single cell (Fig. 103), they are thought to interfere seriously with the normal ciliary action. In consequence, the removal of secretions and inhaled particles is prevented, and the lungs are probably more exposed to infection by inhalation than under ordinary circumstances. The bronchopneumonia which sometimes develops in fatal cases of whooping cough may be due to *H. pertussis* or to other bacteria, such as the pneumococcus.

Bacteriological Diagnosis of Whooping Cough. The bacillus may be isolated by the cough plate method using the potato-glycerol-blood agar of Bordet and Gengou. The open plate is held 4 to 5 inches from the mouth and exposed to one or more explosive coughs. Characteristic colonies appear in two to three days but the plate should be retained for five days before discarding as negative. Nasopharyngeal swab cultures are reported to give a somewhat higher proportion of positive cultures than the cough-plate method.²¹ The



Fig. 103. Whooping cough. Minute bacilli present in masses between cilia of two cells lining the trachea, \times about 1500 (Mallory and Horner).

swab, on thin flexible copper wire, is passed through a nostril into the nasopharynx and left there for two or three coughs before withdrawal and culture on Bordet-Gengou medium. Sputum culture is not particularly satisfactory. Colonies of *H. pertussis* are larger and more opaque than those of the influenza bacillus, it may be differentiated further by hemolysis on blood agar and growth in the absence of the X and V factors.²²

Epidemiology. An upper respiratory infection, whooping cough is transmitted by means of the secretions of the mouth and nose, to some extent through towels, handkerchiefs, hand-to-hand contact and the like, and undoubtedly to a great extent by droplet infection. Undiagnosed and atypical cases may play an important part in the dissemination of the disease. The age incidence is marked with 96 per cent of the deaths in children under five years of age. In 1945, for example, there were 1545 deaths from whooping cough in the registration area of the United States, a death rate almost five times that for the much more dreaded scarlet fever, and there is no doubt that whooping cough is one of the most important killing diseases of childhood. (Fig. 104.) There appears to be some racial difference in susceptibility since the Negro

²¹ Saito, Miller and Leach: Amer. Jour. Pub. Health, 1942, 32:471; Miller, Leach, Saito and Humber: *ibid.*, 1943, 33:839.

²² The laboratory diagnosis of whooping cough is discussed critically and in some detail by Donald Brit. Med. Jour., 1938, Sept. 17, p 613.

against Virus B intranasally, the ratio of respiratory to circulating antibody rose still higher, and the resistance of the animals to intranasal infection with virulent A virus was increased. The effect of the B virus was to cause a local increase in capillary permeability and so help to concentrate the antibody in the respiratory tract. This phenomenon Fazekas de St. Groth refers to—rather portentously—as *pathotopic potentiation*. The applicability of these observations to man must await specific trials.

Numerous attempts were made before and during the second world war to vaccinate against influenza (Francis and Magill 1937, Stokes *et al.* 1937, Horsfall *et al.* 1941a, b, Hirst *et al.* 1942, Burnet 1943, Henle *et al.* 1943, Francis *et al.* 1943, 1944, Report 1944b, 1945). Fluids from infected hens' eggs were mainly used, and the virus was inactivated by a variety of methods. The rise in protective antibody titre after vaccination seemed to be related to the pre-vaccination titre of existing antibodies as well as to the dose and quality of the vaccine; and in general the higher the antibody titre was at the time of exposure to infection, the greater appeared to be the degree of resistance. Immunity took a week or two to establish, lasted for 6 weeks or so, and then began to decline. Many of these early trials yielded very promising results; but trials made later, particularly during and after 1947, were on the whole disappointing. There is reason to believe that this was due mainly to the appearance of epidemic strains differing in their antigenic structure from those previously encountered and incorporated in the vaccine (see Sigel *et al.* 1948, Rasmussen *et al.* 1948, Kalter *et al.* 1948, Taylor 1949). It may prove possible in future to use for vaccine production strains showing epidemic prevalence; but whether this will be successful or not will probably depend on the frequency and degree with which antigenic variation occurs in Nature and the practicability of making the necessary changes with sufficient rapidity to meet the oncoming epidemic. There is, however, some evidence to suggest that the number of possible antigenic variants is limited (Jensen and Francis 1953). If this proves to be true, then the preparation of a satisfactory polyvalent vaccine may become practicable.

Another reason why influenza vaccine has often given disappointing results in practice is that epidemics of influenza are sometimes accompanied by outbreaks of non-influenzal respiratory disease. Should the influenza cases constitute only a small proportion of all cases of respiratory infection, the vaccine cannot be expected to have any substantial effect on the total attack rate.

It must be remembered that, since repeated attacks of influenza may occur under natural conditions, more than a partial degree of transient immunity can hardly be expected to result from vaccination. The immunizing effect of vaccination may perhaps be enhanced by the use of suitable adjuvants (Salk and Laurent 1952), and the necessity of repeated inoculation reduced somewhat. Even if vaccination cannot be relied upon to protect against an epidemic, it may tend to check its progress and diminish its severity by raising the general level of herd immunity. The part it will ultimately play, however, in prophylaxis is very doubtful, and there are those who are frankly sceptical of its value as a measure adaptable to the general population.

Anti-influenzal serum containing a fairly high content of neutralizing antibody was prepared in a horse by Laidlaw, Smith, Andrewes and Dunkin (1935). When injected intraperitoneally into mice, it increased their resistance to the intranasal injection of virus. Passive immunization has, so far, however not been successfully accomplished in man.

death rate is considerably higher than that of the whites. In contrast to most other diseases the incidence in females is somewhat higher than that in males. The seasonal incidence shows a drop during late summer and early fall, with

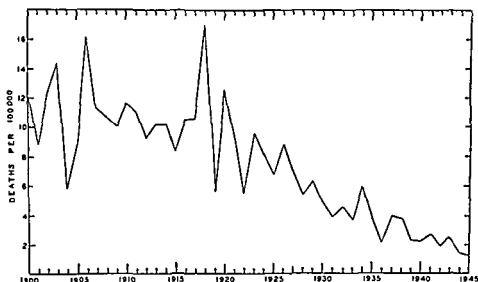


Fig. 104. The prevalence of whooping cough in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

a prolonged peak in the later winter and spring (Fig 105). The disease tends to recur in periodic epidemic waves, presumably a consequence of the accumulation of a new crop of susceptibles (Fig 104).

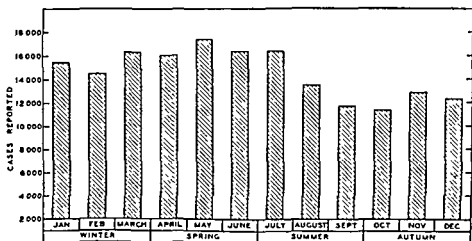


Fig 105. The seasonal incidence of whooping cough. Averages of reported cases by months for the years 1939 to 1945 inclusive. Data from Supplements to Public Health Reports.

Immunity. Recovery from whooping cough is accompanied by the development of an immunity. Second attacks occur only infrequently in children and then are very mild, in older people second attacks are more severe. Com-

brought evidence to show that swine lungworms—*Metastrongylus elongatus* and *Choerostrongylus pudendotectus*—play an important part in the propagation of the disease. Lungworms taken from sick or from convalescent swine have been found to carry the virus, though in a masked form. The infected ova are coughed up by the pig and excreted in its faeces. They are swallowed by earthworms, in which they pass through three larval stages. The third stage is infective for swine. If the earthworm is then eaten by a pig, the larvæ penetrate the intestinal mucosa and migrate to the respiratory tract by way of the lymphatics and blood stream. After two further developmental stages, they finally become adult lungworms and occupy the bronchioles at the base of the diaphragmatic lobes. If infected earthworms are fed experimentally to swine, nothing happens; but if the animals are given multiple inoculations intramuscularly of living *H. influenzae-suis*, some of them develop typical influenza—provided the experiment is carried out during the winter. Both the lungworms and the earthworms may remain infected for several months. The infected earthworms are probably eaten by swine at the time of the late spring and early autumn rains, when the worms come to the surface of the soil. The pig becomes infected in this way with the virus—still in the masked form. If at the time *H. influenzae-suis* happens to be present, then it requires only some circumstance, such as a climatic or physical change—perhaps some weeks or months later—to activate the virus, allow it to penetrate the cells of the respiratory tract, and give rise to typical influenza. It should be mentioned that Shope's work still awaits confirmation in other countries.

Vaccination against swine influenza may be carried out using various types of vaccine, but it is still too early to say what part it will play in the control of the disease (see McLean *et al.* 1947, Gulrajani 1951).

Infectious Pneumonia of Pigs.—It may be well to refer here to a disease which can readily be confused with swine influenza but which appears to be distinct from it. Infectious pneumonia of pigs was established as a separate disease by Pullar (1948, 1949) in Australia. Its existence in England and Northern Ireland was confirmed by Gulrajani and Beveridge (1951), who found it to be far commoner than swine influenza. Infectious pneumonia develops more slowly than swine influenza; pneumonic lesions, which are marginal in distribution, are not evident till the 2nd week. The disease can be transmitted experimentally by the intranasal inoculation of a suspension of lung tissue that has been filtered through a gradocol membrane of 0.8μ A.P.D. The virus cannot be cultivated in the developing chick embryo, nor does it produce illness on intranasal injection into ferrets or mice. Convalescent animals do not contain antibodies to the swine influenza virus in their blood serum.

Influenza in other Animals.

There are several other animal diseases, occurring in sporadic or epidemic form, to which the label "influenza" has been attached; "horse influenza" is an example. In the particular instance of the horse disease there is suggestive, if not conclusive, evidence that it is caused by a filtrable virus; and it is altogether probable that we shall find that several animal diseases are in fact due to infection with a virus of the influenzal type (see Discussion 1935). There is evidence, for example, that the disease of calves variously referred to as infectious pneumonia, calf influenzal pneumonia, and perhaps sometimes as scours, is due to a virus infection. The disease is widespread in Great Britain and in certain parts of the United States. It is characterized by fever, pneumonia, and in the later stages diarrhoea. The case fatality may be as high as 50 or 60 per cent. Animals killed at the height of the disease show a catarrhal enteritis and a bronchopneumonia. The disease can be transmitted by ground-up lung suspensions that have

plement-fixing antibodies are formed but do not appear until the third or fourth week of the disease and, consequently, are of limited diagnostic value. It has been claimed that immunes give an allergic skin reaction to the intradermal injection of pertussis bacilli; the value of this reaction is uncertain.

Prophylactic Inoculation. The early attempts to use vaccines of *H. pertussis* were not uniformly successful. More encouraging results were obtained by Madsen and other Danish bacteriologists in the Faroe Islands. In the United States Sauer and his colleagues have prepared vaccines of freshly isolated, virulent strains which appear to be effective in reducing the incidence and severity of the disease. It appears to be of primary importance that the vaccine consist of the smooth bacilli of Phase I, and earlier reports of the inefficacy of active immunization are probably attributable in part, at least, to the use of other than the smooth form of the microorganism. Important parts of the immunizing antigen are soluble and removed by washing the cells; this material has been partially purified by methanol precipitation in the cold and studied by Pillemer, Burrell and Ross.²³ It is now definitely established that active immunization with properly prepared vaccine confers an effective immunity to pertussis.²⁴ Alum precipitated vaccine seems to be effective also but it is not known whether it is superior to plain vaccine. It appears also that pertussis vaccine may be successfully combined with diphtheria toxoid to allow immunization against the two diseases simultaneously; available evidence suggests that the immune response is equal to that obtained with separate antigens. It is generally agreed that the disease is appreciably milder in immunized children.

With the demonstration of the endotoxins of *H. pertussis*, considerable interest has attached to the possible role of anti-endotoxin in immunity to the disease. The toxicity appears to be neutralized with appropriately prepared antiserum and studies with experimental animals suggest that antitoxic antibody may be of importance in the immunity.²⁵ The results have not yet been applied to man except on a small scale.²⁶

There is some evidence that antiserum may have prophylactic value²⁷ but neither antisera nor vaccines appear to have therapeutic value.

THE MORAX-AXENFELD DIPLOBACILLUS (HEMOPHILUS DUPLEX)

A small bacillus, described independently by Morax and by Axenfeld in 1896 and 1897, is responsible for infections of the conjunctiva and cornea in man, and is known as the Morax-Axenfeld bacillus or *Bacillus lacunatus* (from *lacuna*, a cavity). It has

and in pairs or

²³ Pillemer, Burrell and Ross. *Science*, 1947, 106:36.

²⁴ See the general reviews by Felton and Willard. *Jour. Amer. Med. Assn.*, 1944, 126:294; Lewis. *Med. Officer*, 1946, 76:5; Parish, Gunn and Ungar. *Pub. Health*, 1946, 59:165.

²⁵ See Evans: *Lancet*, 1942, i:529; Anderson and North. *Australian Jour. Exp. Biol. Med. Sci.*, 1943, 21:1; Roberts and Ospeck: *Jour. Inf. Dis.*, 1944, 74:14, Ospeck and Roberts. *Jour. Inf. Dis.*, 1944, 74:22.

²⁶ Bullova and Alterman. *Jour. Amer. Med. Assn.*, 1942, 120:886.

²⁷ Cf. Felton. *Jour. Amer. Med. Assn.*, 1945, 128:26.

about 11 days. The patient is infectious chiefly during this stage and to a less extent during the first week of the paroxysmal stage (see Lawson 1933).

Parapertussis is a disease that resembles pertussis but is usually very mild and of short duration. It is caused by the closely allied organism, *H. parapertussis*. The two diseases do not afford cross-protection to each other.

Diagnosis.

The clinical diagnosis of whooping cough is often difficult and confirmation may be sought from the laboratory. During the catarrhal stage there is usually a leucopenia, but this changes during the paroxysmal stage to a leucocytosis with an absolute and relative lymphocytosis. The constancy of these findings is under dispute, but blood changes may be of help in cases seen too late for cultural methods to be of value.

Cultural.—As has been stated, *H. pertussis* can almost always be isolated from the sputum during the early stages of the disease (see Wollstein 1909, Chievitz and Meyer 1916). For several years the cough-plate method has been extensively used for diagnostic purposes, and has given satisfactory results. The patient is induced to cough directly on to a plate of Bordet-Gengou medium, and this is examined for the characteristic colonies after 48–72-hours' incubation (see Chapter 33).

Among reports on results obtained by this method are the following. Madsen (1925), in 914 cases, records 75 per cent. of positive isolations during the initial catarrhal stage, 61 per cent. during the 2nd week, 45 per cent. during the 3rd, 40.5 per cent. during the 4th, and 9 per cent. during the 5th. Sauer (1933), in 400 cases, records 88 per cent. of positive isolations in the early catarrhal stage, 64 per cent. in the paroxysmal stage, and none in the late stages. Gardner and Leslie (1932), in 47 cases, record 67–75 per cent. of positive results in the first 3 weeks, 25 per cent. in the 4th and none thereafter. Kendrick and Eldering (1934), in 207 cases, record 78–84 per cent. of positive results during the first 3 weeks, 39 per cent. in the 4th and none in the 6th (see also Sugare and McLeod 1929, Sauer and Hambrecht 1930, Kristensen 1933, Straker and Westwater 1937, Donald 1938). It may be added that Kristensen (1933) records his failure to isolate *H. pertussis* from 500 healthy persons, and from 202 persons suffering from other respiratory diseases. Among 301 contacts, *H. pertussis* was isolated in 9 instances, and all these 9 persons subsequently developed whooping cough. Kendrick and Eldering (1934) and Wilcox (1934) also record their failure to isolate the bacillus from any disease other than whooping cough.

The incidence of *H. parapertussis* varies greatly. In Michigan Eldering and Kendrick (1952), using mainly the cough-plate method, isolated *H. pertussis* from 3,263 and *H. parapertussis* from 65 patients during the years 1935–50; that is, *H. parapertussis* was responsible for only about 2 per cent. of the cases diagnosed as whooping cough from which diagnostic cultures were made. In Great Britain this organism is likewise uncommon, but in Denmark its proportional frequency has at times been considerably greater.

To avoid some of the inconveniences of the cough-plate method, Maclean (1937) introduced the use of the *post-nasal* or nasopharyngeal swab. Cultures made by this method are liable to be overgrown by other organisms but, if the swab is streaked on to a Bordet-Gengou plate over which a few drops of a suitable strength of penicillin have been spread, these can be largely suppressed. An even better method is to use a *pernasal* swab (Bradford and Slavín 1940, Brooks and Bradford 1942, Miller *et al* 1943, Cockburn and Holt 1948). This consists of a thin wisp of wool wound round the end of a length of fine flexible nichrome wire. It is

short chains. They are non-motile, non-spore-forming and gram-negative. They do not grow on the ordinary nutrient media, potato, milk or gelatin but require the presence of serum, ascitic fluid or blood in the culture medium. They ferment few if any carbohydrates and do not form indol. Coagulated serum is liquefied. They die out within a day or two at room temperature but may survive for weeks in culture in the incubator. It has been reported²⁸ that hemolytic and non-hemolytic types occur which may be differentiated immunologically.

So far as is known this microorganism is pathogenic only for the human eye. The inoculation of experimental animals is without effect, but the instillation of the bacilli onto the conjunctival sac of man results in the development of a blepharoconjunctivitis, either chronic or acute, and severe inflammation of the cornea may be produced. Treatment with 0.25 per cent zinc sulfate solution is specific and produces a rapid cure, while silver salts are without effect. The



Fig 106. Morax-Axenfeld diplobacillus, smear taken from conjunctiva (Brown Pusey).

disease is widely distributed and has been reported in Europe, Africa and North America.

DUCREY'S BACILLUS (HEMOPHILUS DUCREYI)

"Soft chancre" or "chancroid" is a venereal disease transmitted by direct contact. The lesions, which are on the genitals or adjacent areas, are irregular ulcers which differ from the hard or hunterian chancre—the primary lesion of syphilis—in that they are not indurated. Unlike syphilis, the infection remains localized, spreading no further than the neighboring lymphatics, which may become swollen to form secondary buboes in the groin.

The bacillus which bears his name was found by Ducrey in 1890 in the purulent discharge from the lesion, and by the inoculation of the skin of the forearm he was able to transmit the disease through fifteen generations. The microorganism was obtained in pure culture by Besançon, Griffon and le Sourd in the same year.

Ducrey's bacillus is a short rod 1 to 1.5 μ in length and 0.6 μ in breadth. In smears it is generally found to be ovoid, and there is a tendency to occur in end-to-end pairs or in short chains; in broth culture longer chains may be observed. It is non-spore-forming and nonmotile. The bacillus not infrequently

²⁸ Oag. Jour. Path. Bact., 1942, 54:128.

immunization with an *H. pertussis* vaccine is only moderately effective in preventing the disease, but is of very real value in decreasing severity and mortality.

Sauer (1937) used a plain vaccine with apparent success in routine clinic practice with healthy infants. Kendrick and Eldering (1936, 1939), likewise using a plain vaccine, obtained a considerable degree of protection in a controlled study in Michigan; the incidence of whooping cough in the vaccinated children was only about a third of that in the controls. Bell (1941, 1948) obtained similar results in two well controlled trials, the first made with an alum-precipitated vaccine alone, the second with an alum-precipitated vaccine combined with diphtheria prophylactic. An alum-precipitated vaccine tried by Kendrick (1942, 1943) also proved successful. Other favourable reports were made by Rambar and his colleagues (1941), Daughtry-Denmark (1942), Perkins and his colleagues (1942) and Sako (1947) in the United States, by Oláh (1942) in Hungary, and by Dungal, Thoroddsen and Ágústsson (1944) in Iceland. On the other hand, Siegel and Goldberger (1937) and Doull and his colleagues (1939) in the United States, and McFarlan, Topley, and Fisher (1945) in England failed to obtain any substantial degree of protection in controlled field trials among infants and children.

It should be pointed out that in some of the studies referred to above there was no control group, and that in others the method of selecting the controls was not above criticism. In mass immunization experiments too much care cannot be taken to ensure that the treated and the control subjects are as similar as possible in every respect. The fallacy of offering immunization to a given group of subjects, inoculating those who accept and treating the remainder as controls, has already been pointed out in relation to B.C.G. vaccination (p. 1531). It is particularly difficult in clinic practice to be sure that children in the two groups are exposed to equal risks of infection. The most reliable method, therefore, is to inoculate alternate children in a residential nursery or similar institution, in which all the children are mixed together and in which infection, if it is introduced, will spread to all alike. This method, however, is usually practicable for use on only a small scale.

To satisfy the most rigorous conditions a field trial was carried out in England by the Whooping-Cough Immunization Committee of the Medical Research Council (Report 1951). Infants and children of 6-18 months of age whose parents agreed to take part in the trial were divided by random sampling into two groups of equal size. One was given 3 doses at monthly intervals of pertussis vaccine, the other of an anti-catarrhal vaccine. The pertussis vaccine included 5 batches from 3 different manufacturers. Each child was visited frequently by a special nurse, and swabs were taken for bacteriological diagnosis from clinical cases of whooping cough. Altogether 10 trials were carried out in 5 separate areas. A total of 3,801 children injected with the pertussis vaccine and 3,757 injected with the control vaccine were followed up for a period of 2-3 years. The average attack rate in all the trials taken together was 1.45 per 1,000 child-months of observation in the vaccinated and 6.72 in the control group. The best comparative index proved to be the attack rate among children under 14 years of age exposed to risk of infection in their own homes. These rates were 18.2 per cent. in the vaccinated and 87.3 per cent. in the control group. The vaccines varied in their protective power. With the best an attack rate among home exposures of only 7.3 per cent. was recorded, with the least effective the corresponding figure was 30.4 per cent. This trial showed without doubt that it is possible to confer substantial protection against whooping cough by vaccination, and that different vaccines vary in their protective potency.

stains irregularly and bipolar staining may be observed. It is stained by the usual aniline dyes and is gram-negative.

The bacillus will not grow on the ordinary laboratory media and requires the addition of serum or, preferably, blood. According to Beeson²⁹ the X and V factors alone are not sufficient and additional factors present in either serum or erythrocytes are required. The small, grayish, glistening colonies appear in blood agar in twenty-four hours and, after two to three days' incubation, show a narrow zone of hemolysis. The bacilli may be cultivated from the chancroid by inoculating tubes of fresh (not more than three to five days old) rabbit blood. Smears made at the end of twenty-four to forty-eight hours' incubation will show the characteristic tangled chains of gram-negative bacilli.³⁰ These bacteria cannot be identified in smears from the chancroid because of a tendency to aberrant morphology.³¹ The bacilli may be isolated and cultivated on the chorioallantois of the developing chick embryo, and appear to have little pathogenicity for the embryo.³² Rabbits and monkeys may be infected by intradermal inoculation of pure cultures, but subcutaneous, intraperitoneal and intravenous inoculation is without effect.³³ Infected animals develop a hypersensitivity but no immunity to reinfection.

There is little or no immunity. The chancroid is frequently multiple and is auto-inoculable. A hypersensitivity develops, however, which is manifested as a reaction to the intradermal inoculation of killed bacilli, and which persists for many years. Antisera and autogenous vaccines are said to have therapeutic value.

²⁹ Beeson: *Proc. Soc. Exp. Biol. Med.*, 1946, 61:81.

³⁰ Beeson and Heyman: *Amer. Jour. Syph.*, 1945, 29:633.

³¹ See the discussion of diagnosis by Greenwald. *Jour. Amer. Med. Assn.*, 1943, 121:9

³² Anderson and Snow: *Amer. Jour. Path.*, 1940, 16:269.

³³ See the discussion by Feiner, Mortara and Levenkron: *Amer. Jour. Syph.*, 1945, 29:71.

opinion The arguments are set out in some detail in the report of a special conference held under the auspices of the World Health Organization, to which reference may be made (Report 1953a). Our own preference is to delay vaccination against both whooping cough and diphtheria till towards the end of the first year of life, partly to ensure the highest degree of protection during the pre-school years when the risk of developing these diseases is greatest, and partly to avoid the necessity of a re-inforcing injection till the child enters school. Whether a re-inforcing injection is necessary for whooping cough is very doubtful, but it certainly is for diphtheria. A combined vaccine has obvious advantages. We know, however, too little about the resulting immunity to whooping cough to recommend such a vaccine unreservedly (see Barr and Llewellyn-Jones 1953). Moreover, there is some evidence to suggest that poliomyelitis is more likely to follow the use of a combined than of a simple vaccine.

Complications.—Encephalitis is a rare complication of whooping cough. Convulsions and encephalopathy have been described after pertussis vaccination (Byers and Moll 1948, Toomey 1949, Anderson and Morris 1950)—mainly in infants and children showing signs of mental instability. Poliomyelitis, on the other hand, is seen after vaccination in perfectly healthy children. Since McCloskey (1950, 1951) drew attention to this sequela in Australia, several observations have been made on its incidence, with varying results. If consideration is restricted to cases coming on within 4 weeks of inoculation and affecting the inoculated limb first, there does seem to be, in some countries and at some times, a greater degree of association between inoculation and paralysis than can be explained by chance alone (see Hill and Knowelden 1950). The risk appears to be greater after combined than after simple vaccines. It is probably wise therefore to discontinue inoculation temporarily in areas where poliomyelitis is epidemic, or to avoid at such times the use of combined vaccines.

Treatment.—Since about 70 per cent. of deaths occur from whooping cough before the age of one year, and since it is often impracticable to confer active immunity on all infants by vaccination, even if this was desirable, recourse has to be made to the use of therapeutic agents. Antisera can be prepared against the exotoxin of *H. pertussis*, but these appear to have little effect on the course of the human disease (Bullowa *et al.* 1942). There is some evidence to suggest that serum from convalescent cases may be of value when given prophylactically during the incubation period or therapeutically in the early catarrhal stage, but further observations are required (see Smith 1936). Most deaths from whooping cough in infancy occur as the result of respiratory complications, particularly bronchopneumonia. These are often susceptible to treatment with the sulphonamides or the antibiotics. Chloramphenicol is said to shorten the course of the disease (Lassen and Grandjean 1951), though this effect seems to be restricted to patients treated within the first week (Report 1953b). It is, however, too dangerous a drug to use, and aureomycin should be substituted for it if antibiotic treatment is regarded as desirable.

Secondary Broncho-Pneumonia

Influenza, whooping cough, measles, and to a less extent certain other specific infective diseases, are peculiarly liable to be associated with a secondary infection of the lungs; and it is this fact which is answerable for the high mortality associated with such diseases. The case fatality during an epidemic of influenza, for instance, is almost entirely determined by the frequency of pulmonary complications.

PSEUDOMONAS; LACTOBACILLUS,
LISTERIA; BACTEROIDES; BARTONELLA;
PLEUROPNEUMONIA GROUP, DONOVANIA

PSEUDOMONAS PYOCYANEA

The genus *Pseudomonas* includes some thirty species which are found, for the most part, in water, soil and wherever organic matter is decomposing. The fluorescent bacteria are members of this genus, *Ps. fluorescens* being the gelatin-liquefying form and *Ps. non-liquefaciens* the non-liquefying form. The elaboration of blue pigment by *Ps. synchyanea* results in "blue milk." One species, *Ps. septicæ*, is the cause of a disease of caterpillars, another, *Ps. jaegeri*, is pathogenic for chickens, and a third recently discovered species, *Ps. reptilovorans*, produces disease in certain reptiles.

The best known species of *Pseudomonas*, however, and the only one that is pathogenic for man, is *Ps. pyocyanea*. The blue or blue-green stains that sometimes appear upon surgical dressings long ago attracted attention, and even before the cause of the phenomenon had been discovered Fordos studied the pigment in 1860. Gessard found, in 1882, that the pigment was the product of a specific microorganism, *Ps. pyocyanea*, which he isolated in pure culture.

Morphology and Staining. The cells of *Ps. pyocyanea* vary considerably in size and proportion, but appear usually as small, slender rods, 1.5 to 3 μ long and 0.5 μ broad, frequently united in pairs and short chains. There are 1 to 3 polar flagella, and the bacterium is actively motile. Neither capsules nor spores are formed. The colonies are large and spreading, edges are irregular, and the consistency butyrous. These bacilli stain readily with the usual aniline dyes and are gram negative.

Physiology. *Ps. pyocyanea* grows readily on all the ordinary culture media and most rapidly at a temperature of 30° to 37° C. Aerobic conditions are required, although it is sometimes said that there is some growth under anaerobic conditions. Gelatin is rapidly liquefied, hydrogen sulfide is produced, and indol formation is variable. The fermentative abilities of this bacterium

One of the most distinctive characteristics of *Ps. pyocyanea* is its production of a bluish green, soluble pigment which does not color the colonies or other parts of growth. The pigment is actually two colors, blue and green, and can be extracted by boiling in water, yielding a yellowish green

In many infections by the organisms mentioned in this section, the disease may, on both clinical and pathological grounds, be classified as a primary pneumonia. In others, the distinction between primary and secondary pneumonia is hard to make and, from many points of view, may not be worth making. Nevertheless, the term "secondary pneumonia" at least serves to remind us of the patient's predisposition to bacterial respiratory infections in diseases like measles and influenza; and from the foregoing it is clear that pneumococci, influenza bacilli, hæmolytic streptococci and staphylococci are the chief offenders in taking advantage of this predisposition, together accounting for the majority of fatal infections.

The fact that these secondary pneumonic infections play a large part in determining the case-fatality rate in any outbreak of influenza, measles or whooping cough, raises a point of the first importance in regard to the effective control of these diseases. It is probable that the secondary bacterial invader responsible for the fatal issue is in many cases leading a vegetative existence in the upper part of the respiratory tract of the host, at the time when the primary infection is contracted (see Smillie and Duerschner 1947). In other cases the host may receive the secondary invader from the same source, and at the same time, as the primary infecting agent. There is, however, little doubt that a considerable proportion of fatal secondary infections result from the implantation, on a case of primary influenza or measles, of a virulent streptococcus or pneumococcus; and the records contain instances of waves of secondary infection, due to a particular bacterial species, passing through hospital wards or concentration camps (see Opie *et al.* 1921). The conditions which, in the aggregate, make up the environmental factor known as "hospitalization," play the same part in secondary respiratory disease as they play in the case of surgical or puerperal sepsis. The patient with influenza or measles should be regarded as peculiarly liable to acute respiratory infection, and should be carefully guarded from secondary infection. There is, for instance, ample evidence that overcrowding in hospital wards, or in military camps, may seriously increase the mortality during an outbreak of influenza or of measles (see Memorandum 1944).

There seems little hope that any form of prophylactic immunization, apart from immunization against the primary disease, will reduce the liability to such secondary infections. As will be obvious, we are faced with a multiplicity of heterogeneous bacterial antigens, and it seems scarcely reasonable to expect that we can ever induce immunity to all the bacterial species and types to which a person with some primary predisposing infection may become a prey. The same heterogeneity of infecting agents severely limits the opportunities for the use of specific serum therapy.

Both the sulphonamides and penicillin are reported to be effective in the control of broncho-pneumonia due to staphylococci and streptococci. The sulphonamides are less effective against *H. influenza* and *Bact. friedländeri*, though some success is claimed for them.

Primary Pneumococcal Pneumonia

We need not here consider the clinical or pathological aspects of pneumonia as it occurs in man, or discuss the vexed question of the classification of acute inflammatory lesions of the lungs. Excluding the broncho-pneumonias of young children, and the secondary pneumonias to which we have already referred, there

fluorescent pigment, is soluble in water but not in chloroform. They are both oxidation products of colorless precursors. Both sulfate and phosphate are required for the formation of pyocyanin. Pyocyanin is formed only by *Ps. pyocyanea*, but the fluorescent pigment is formed by several other species of *Pseudomonas*. These pigments may occur separately or together, and the conditions that determine their formation have been exhaustively studied¹ Wrede² has determined the composition of pyocyanin, which has proved to be an entirely new type of dye and the first instance of a phenazine derivative occurring in nature (p. 124).

Pathogenicity. For some time after its discovery *Ps. pyocyanea* was generally regarded as a harmless saprophyte, or at the most as a microorganism of slight pathogenic power. It has since been learned that this bacterium is causally associated with a great variety of suppurative and other affections in

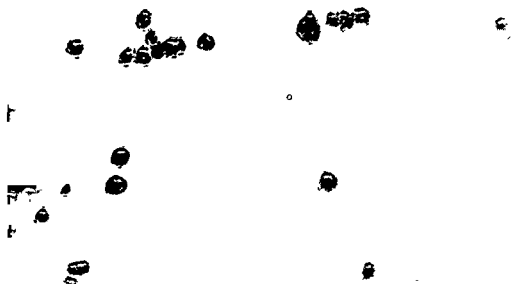


Fig. 107. Colonies of *Ps. fluorescens* on nutrient agar. Twenty-four-hour culture, $\times 3$.

man. Apart from the many doubtful cases in which *Ps. pyocyanea* is found mixed with streptococci, staphylococci and other microorganisms where its share in inciting pathological processes is problematic, numerous instances are on record in which little or no question exists as to its etiological role. It has been found by a number of workers in pure culture in abscesses in different parts of the body, especially in the middle ear. Cases of endocarditis and pneumonia have also been met in which *Ps. pyocyanea* seemed to be the sole responsible microorganism. A generalized and fatal form of pyocyanic infection has been observed by a number of investigators, and the bacillus has been found in the blood during life. *Ps. pyocyanea* has been found constantly present in the intestinal discharges of patients during a dysentery-like epidemic, and the same microorganism was also present in abundance in drinking water which seemed, on epidemiological grounds, to be implicated in the outbreak. Ghosh³ reported a series of cases of infection with this bacillus that closely

¹ Jordan: Jour. Exp. Med., 1899, 4:627.

² Wrede. Ztschr. f. Hyg. u. Infektionskr., 1930, 111:90

³ Ghosh: Jour. Indian Med. Assn., 1938, 7:655

by pneumococci and Friedländer's bacillus, but is less fatal (Glaser and Wood 1951). The mechanism of recovery from experimental pneumonia under chemotherapy was studied in rats by Wood and his colleagues (for references see Sale and Wood 1947).

It should be noted that the action of the starch or mucin in these experiments is not to block the bronchi and thereby induce collapse and subsequent infection in the tributary alveoli, but to protect the cocci against the defence mechanisms of the body (see Chapter 54). According to Hamburger and Robertson (1940) mere blocking of a bronchus does not predispose the corresponding alveolar portion of the lung to infection. It is probable that natural infections are initiated by the aspiration of pneumococci and mucinous material from the upper respiratory tract. Nungester and his colleagues, for example, have shown in rats that the *nasal* instillation of pneumococci and mucin does not result in pneumonia but that, if by chilling or alcohol intoxication the normal mechanism preventing the inhalation of fluids into the lung is deranged, pneumonia follows (Nungester and Klepser 1938). When mucin and pneumococci were introduced into the nose or the trachea, artificially imposed forceful inspiration induced pneumonia in about 50 per cent. of the rats tested. Forceful expiration, on the other hand, which in some respects is analogous to natural expulsive coughing, had a smaller predisposing action, for, when it was imposed on the breathing of mice inoculated intratracheally, the incidence of pneumonia was only 18 per cent. (Nungester, Klepser and Kempf 1942).

As noted elsewhere, Gaskell (1927) carried out a large series of intratracheal inoculations in rabbits, and brought evidence to show that the type of infection produced in these animals is mainly determined by the virulence of the strain of pneumococcus injected, ranging, as virulence decreases, from a rapidly fatal septicæmia without pulmonary changes, through lobar and lobular pneumonia, and through lesions confined almost entirely to the bronchi and bronchioles, to a complete absence of reaction. The frequency with which pneumonia follows intranasal inoculation in mice has been found to vary both with the virulence of the strain and with the susceptibility of the animal used (see Rake 1936).

Though consideration of the epidemiology of pneumonia in man points overwhelmingly to the respiratory route of entry, the possibility of a blood-borne infection of the lung must be borne in mind. In this connection we may note that Rake (1936), by carefully balancing the virulence of the pneumococci against the resistance of the test mice, induced pneumonia by intravenous inoculation; and that Kempf and Nungester (1940) showed that pneumonia followed intravenous inoculation in rats previously given mucin intrabronchially.

If we collate the available experimental evidence with the numerous observations which have been carried out on the natural disease as it occurs in man (Dochez 1912, Sia, Robertson and Woo 1928, Finland and Sutliff 1931a), we can construct a picture of the sequence of events in lobar pneumonia, which, however incomplete it may be, will aid us in the discussion of the results obtained in serum treatment and in prophylactic vaccination.

It would appear that the initial phase of the infection is marked by a progressive invasion of the lung tissue, frequently associated with a detectable bacteræmia. The inflammatory reaction in the lung progresses through its successive stages; and the bacteræmia may, or may not, increase in severity. At some time between the 4th and 7th days, in favourable cases, specific antibodies begin to appear in the blood; and, coincidentally with their appearance, the temperature falls by crisis, the pneumococci disappear from the blood if they were ever present in detectable numbers, and the clinical condition of the patient undergoes a striking amelioration, though abnormal signs in the lungs may persist for several days or even weeks.

There are good grounds for believing that the degree and persistence of bacteræmia, rather than the local condition in the lungs, is the main factor in determining

simulated Asiatic cholera. There is no doubt that under certain conditions *Ps. pyocyanea* is pathogenic for man, although probably human infections are relatively rare.

The intraperitoneal injection of 0.25 ml. of culture of a virulent strain will kill a guinea pig with acute symptoms in twenty-four hours. Smaller amounts are also fatal but less rapidly. Subcutaneous inoculation produces a marked local reaction. The symptom complex presents nothing especially characteristic. Rabbits are not so susceptible as guinea pigs, mice and pigeons are less susceptible than rabbits. Immunity can be produced by small, non-lethal doses.



Fig. 108. *Lactobacillus* sp. isolated from the mouth. Morphologically identical with *L. acidophilus*. Note the diplobacillary form and palisade arrangement of the cells. $\times 2500$ (Harrison).

The occurrence of *Ps. pyocyanea* as a plant pathogen and its close relationship, if not identity, with *Phytomonas polycolor*⁴ has been noted elsewhere (p. 328).

LACTOBACILLUS

This genus is composed of a somewhat loose assemblage of microorganisms which produce considerable quantities of lactic acid from carbohydrates and which are able to withstand a degree of acidity usually fatal to non-sporulating bacteria. The latter characteristic is useful in the isolation of cultures as well as in distinguishing the group. Kendall has proposed a term, *aciduric* (acid tolerant), which is now commonly applied.

Morphologically some of the lactobacilli are long, slender rods, while others are somewhat similar to the colon bacillus but unlike it they are all gram-positive. They are non-motile. Many cultures exhibit a typical diplobacillary form, often kidney-shaped. Old cultures frequently exhibit considerable pleomorphism. As a rule, cultures are facultatively anaerobic or microaerophilic, and some of them, particularly *L. bifidus*, are anaerobic on primary isolation.

Classification has been based primarily upon source of the cultures. Al-

⁴Elrod and Braun. Jour. Bact., 1942, 44:633.

rates were progressively less with Type II, Type I and Group IV infections, in that order (see Glynn, Digby and Jones 1923, Cecil 1928, Rosenblüth 1928, Ferguson and Lovell 1928).

The differentiation of Group IV strains into multiple types has not materially affected the picture, though it has painted in a little more detail. Most of the new types are met with only occasionally; Types 6, 7, 14 and 19, however, may sometimes rival the older types in frequency. On the whole, the newer types tend to be found relatively more often in secondary pneumonias than in primary lobar pneumonia. The type distribution in the pneumonias of childhood may differ considerably from that in adults (see Nemir *et al.* 1936); and the types associated with disease in different parts of the body, such as the middle ear, the pleura, or the spinal fluid, often have characteristic distributions. In healthy carriers the type distribution differs from that found in lobar pneumonia, but often corresponds closely to that found in broncho-pneumonic infections (see Finland 1937). Table 149 records representative figures taken from Finland's (1942) review showing the

TABLE 148

FREQUENCY OF THE DIFFERENT SEROLOGICAL TYPES OF PNEUMOCOCCI IN LOBAR PNEUMONIA AS RECORDED BY VARIOUS OBSERVERS.

Observers.	Country or District.	Period.	No. of Cases examined.	Frequency percentage.			
				Type I.	Type II.	Type III.	Group IV.
Schorer, Clark and others	U.S.A	1917-18	101	30.7	14.9	17.8	36.6
Thomas	U.S.A.	1917-21	239	25.1	10.9	14.6	49.4
Cecil, Baldwin and Larsen	U.S.A.	1921-26	1,913	33.6	19.1	13.3	33.3
Griffith	England	1920-22	150	30.6	32.7	6.7	30.0
Glynn, Digby and Jones .	England	1919-21	96	45.8	24.0	2.1	28.1
Ferguson and Lovell . .	(Liverpool) England (Manchester)	1925-27	116	43.1	4.3	0.0	52.6

frequency of the Cooper types in pneumonia of adults and of children in the United States of America, and in healthy carriers surveyed by Gundel (1933) in Germany and by Straker, Hill and Lovell (1939) in England (For records of type distributions in various other parts of the world, see Mulder 1938, Ordman 1938, Lal and Chitkara 1941, Stobo and Little 1941, Siegenbeek van Heukelom and Beunwkes 1942, Guthrie and Montgomery 1948, and Lund 1949)

We can divide pneumococci into the relatively virulent or "infective" types, and the apparently less virulent, characteristically "carrier" types. The infective types are commonly associated with acute pneumococcal pneumonia, and the carrier types are commonly found in the healthy human throat. This arbitrary division is not clear cut; as Table 149 shows, "carrier" types may cause severe pneumonia, and "infective" types are carried by healthy persons.

Carrier types are established in the throat in early life (see Sutliff and Davies 1937) and are found there at all ages. Carriage of any one type may persist for years (Straker *et al.* 1939), though the type may be detected only intermittently. Several types may be carried by one person; up to seven have been recorded. In a healthy community

though this is an insecure criterion for differentiation, no really satisfactory basis of classification has been developed. A number of investigators have attempted classifications by means of carbohydrate-fermentation reactions. Many strains otherwise apparently identical differ, however, in fermentative reactions, and many cultures change in reaction after prolonged artificial cultivation. These variations make it doubtful whether such reactions are of any more value than morphological and colonial characters which are also subject to considerable variation. Efforts to classify the lactobacilli on the basis of metabolic activity have been more promising.

Bergey (1948) recognizes fifteen species, differentiated on a physiological basis, viz.,

- (I) Homofermentative, i.e., producing lactic acid primarily.
 - (A) Optimum temperature of 37° to 60° C.
 - (1) Acid from lactose
 - (a) Optimum temperature 37° to 45° C.
 - (i) Produce levo lactic acid
 - Lactobacillus caucasicus*
 - Lactobacillus lactis*
 - (ii) Produce inactive or dextro lactic acid
 - Lactobacillus helveticus*
 - Lactobacillus acidophilus*
 - Lactobacillus bifidus*
 - (b) Optimum temperature 45° to 62° C., usually maltose not fermented
 - Lactobacillus bulgaricus*
 - Lactobacillus thermophilus*
 - (2) Lactose not fermented
 - Lactobacillus delbrueckii*
 - (B) Optimum temperature 28° to 32° C.
 - (1) Acid from lactose
 - (a) Produce dextro lactic acid
 - Lactobacillus casei*
 - (b) Produce inactive lactic acid
 - Lactobacillus plantarum*
 - (2) Lactose not fermented
 - Lactobacillus leichmannii*
- (II) Heterofermentative, i.e., producing considerable amounts of products other than lactic acid, viz., carbon dioxide, acetic acid, alcohol.
 - (A) Optimum temperature 28° to 32° C.; usually ferment arabinose
 - (1) Raffinose not fermented, sucrose and lactose usually not fermented
 - Lactobacillus brevis*
 - (2) Ferment raffinose, sucrose and lactose
 - Lactobacillus buchneri*
 - Lactobacillus pastorianus*
 - (B) Optimum temperature 35° to 40° C. or higher; arabinose usually not fermented
 - Lactobacillus fermenti*

The serological relationships of a number of species have been studied by several investigators. Harrison and co-workers,⁵ using strains of oral origin, studied their polysaccharide precipitation reactions in an effort to establish an immunological classification. They reported the existence of four or more immunological types but indicated later that some strains are unstable, changes in

⁵ Harrison *et al.*: Jour. Inf. Dis., 1939, 65-255, *ibid.*, 1942, 70.69, *ibid.*, 1942, 70.77, Jour. Dent. Res., 1944, 23 1.

As regards the infective types, though it is possible that a persistent carrier may succumb to infection with his own type, there are many examples of epidemic outbreaks in which the responsible pneumococcus apparently infects from without; that is, in which acute lobar pneumonia spreads by contagion. When we study the carrier rates for the infective types we find, in accord with the notion of their relatively high virulence, that high rates are associated with outbreaks of pneumonia. Thus in institutional outbreaks of Type I pneumonia, Type I carrier rates of 10 and 30 per cent. have been recorded (Stillman 1916, Strom 1932). Dauer and others (1941) observed that in a group of 50 boys, five of whom had a Type II pneumonia, the healthy carrier rate for Type II was 44 per cent. It is clear that "infective" types can spread through a community. Indeed Andrews (1937), in a survey of pneumonia of infants due to a wide variety of types, found that the types associated with the highest incidence of pneumonia in infected persons, and with the highest case fatality, were also most frequently isolated from the throats of family contacts.

The relation of the carrier state and prevalence of pneumonia is well illustrated in Smillie and Jewett's (1940) survey of familial outbreaks of pneumonia. They found that, whereas the carrier rate for carrier types like XIX or XXVII was similar for contacts and non-contacts of Type I and Type II pneumonias, the Type I carrier rate in Type I contacts was 10 per cent., but only 0.8 per cent. in non-contacts; in Type II outbreaks the corresponding Type II carrier rates were 7.4 per cent. and 0.3 per cent. (see also Rogers *et al.* 1941, 1943).

It is noteworthy that high carrier rates are often found simultaneously with outbreaks of pneumonia, and that a high carrier rate for a given type may even precede an outbreak (Stebbins *et al.* 1940, see also Smillie and Jewett 1942). Acute lobar pneumonia is but one manifestation of the spread of a virulent pneumococcus; other observed manifestations are the healthy carrier state, infection of the upper respiratory tract, conjunctivitis, otitis media, and mild atypical pneumonia (see Gilman and Anderson 1938, Plummer and Ensworth 1941). The concentration of carriers, as in infections by other bacteria, to some extent determines the appearance of manifest disease. Thus, in the school outbreak recorded by Dauer and others (1941), pneumonia appeared in the group of boys with a 44 per cent. carrier rate, but not in groups with lesser rates (see also Mackenzie *et al.* 1940). This relation, however, is by no means found consistently. Stebbins and his colleagues (1940), for example, record an epidemic prevalence of a Type V pneumonia in the absence of a continued high carrier rate, and in another population a high carrier rate of Type VI without the appearance of Type VI pneumonia.

The available evidence, then, indicates that acute lobar pneumonia is a contagious disease, which is spread by carriers and by persons with a variety of pneumococcal infections of the respiratory tract, including acute lobar pneumonia itself. Certain types of pneumococcus appear to infect more readily than others, but we have little precise knowledge of the circumstances that determine infection of the lung in a given person.

The Treatment of Pneumonia with Antipneumococcal Serum.

In between the two world wars antiserum provided the only specific means of combating pneumococcal infection. With the introduction, however, of the sulphamides and of penicillin, the cumbersome and not very effective method of treatment by antiserum rapidly passed into disuse and for all practical purposes is now merely of historical interest. We do not propose therefore to give more than a brief outline of the method. Those of our readers who are interested may be referred to an analysis of the results that was included in the 3rd edition of this book (pp. 1675-81).

The prophylactic and therapeutic value of antiserum was demonstrated in

immunological specificity occurring apparently in association with changes in fermentation reaction. Application of the agglutination reaction to the serology of the lactobacilli is hampered by a strong tendency to spontaneous agglutination, and earlier workers observed a marked serological heterogeneity. Williams,^{5a} however, using filtered antigens and cross agglutinin absorption, has demonstrated the presence of four well-defined agglutinogens, designated A, B, C and D, whose distribution corresponds closely with the polysaccharide types of Harrison. There is evidence that cultures from the intestine include types identical with those obtained from the mouth.

Physiology. The lactobacilli are microaerophilic or anaerobic on primary isolation but after continued cultivation some strains will grow in the presence of air. Their nutritional requirements are complex and most strains cannot be cultivated on the usual nutrient or infusion media unless these are enriched by the addition of glucose or whey. These requirements have been studied in some detail and lactobacillus strains are frequently used for the microbiological assay of vitamins. For example, Dunn, Shankman and their co-workers,⁶ in a study of the amino acid and vitamin requirements of twenty-three representative strains, found that individual requirements for amino acids varied from two to fifteen, and that pyridoxine, thiamine, riboflavin, biotin, folic acid, pantothenic acid and nicotinic acid are required in general, individual requirements, of course, differing.

Pathogenicity. Aside from a relation to dental caries (see below), the lactobacilli are generally regarded as non-pathogenic and are of interest largely in the dairy and fermentation industries. There are, however, reports in the literature of endocarditis caused by lactobacilli and febrile conditions apparently causally associated with a bacteremia.⁷ These are perhaps a consequence of invasion from a dental lesion, a bacteremia may not be uncommon but pathological consequences seem to be very rare. The intestinal lactobacilli are a part of the normal flora as noted elsewhere and bear no relation to diarrheal disease.

Lactobacillus Acidophilus. First cultivated by Moro in 1900 from the feces of infants, this organism has been isolated from the intestine of nearly all the mammalia, many other vertebrates and some of the invertebrates. It increases in the intestine when the carbohydrate content of the diet is increased and may become predominant when a milk diet is administered.⁸ These bacilli, fairly large and of variable length, are arranged singly, in pairs frequently slightly bent at the juncture, and in palisades (Fig. 108). Long chains, filamentous forms and club shapes are not uncommon. Young cultures stain uniformly gram positive. Old cultures, however, often show beading or bipolar staining and may be easily destained so that they appear to be gram negative. Colonies, usually small, may vary in shape between a smooth, rounded opaque form and a flattened, translucent, irregular form often having a ground glass appearance. Fermentation reactions are variable.

^{5a} Williams, *Jour. Inf. Dis.*, 1948, 52: 31.

⁶ Dunn, Shankman et al., *Jour. Biol. Chem.*, 1947, 168: 1, 23.

⁷ Buera and Seppilli, *Arq. Biol. (São Paulo)*, 1944, 25: 143. *Jour. Inf. Dis.*, 1947, 51: 112.

⁸ Rettger and Chaplin, *A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of Bact. Acidophilus*. New Haven, 1933.

Chemotherapy of Acute Lobar Pneumonia.—Since the demonstration of its efficacy in lobar pneumonia by Evans and Gaisford (1938), sulphapyridine or one of the other sulphonamides has been used widely and successfully in the treatment of all kinds of pneumococcal infections. As we have seen in Chapter 54, there is every reason to believe that different processes are concerned in the antibacterial action of sulphonamides and of specific antibody. We might therefore on *a priori* grounds expect that the two together would be more effective than either alone. On this subject, however, both the experimental observations in animals and the natural observations on human patients yield rather discrepant results. In some instances a synergic effect appears to have been demonstrated: in others the addition of the serum seems to have been without benefit (see Finland and Brown 1939b, Wright and Gunn 1940, Finland 1942). The results obtained with sulphonamides alone, or with penicillin, are now so good that antiserum, though theoretically valuable in bacteraemic cases, is in fact no longer used.

Mention should perhaps be made of the *enzyme treatment* of infections caused by Type III pneumococci. Antipneumococcal serum against this type proved ineffective, but promising results were obtained with a bacterial enzyme prepared by Dubos and Avery (1931) that acted on the specific polysaccharide. A curative action was demonstrated in mice, rabbits, and monkeys, but the treatment does not appear to have been extended to human beings (see Goodner *et al.* 1932, Francis *et al.* 1934, Dubos and Bauer 1935).

Prophylactic Immunization against Pneumonia.

The records of antipneumococcal immunization in laboratory animals are copious, and their significance is unequivocal. It is clear that susceptible animals, such as mice and rabbits, can be immunized with almost uniform success against infection by the usual routes. More immediately pertinent, perhaps, are the observations of Cecil and Blake (1920*a, b, c*) and Cecil and Steffen (1923, 1925), which showed that monkeys could be immunized with a considerable measure of success against intratracheal infection with Type I or Type II pneumococci, though the results with Type III were less promising (see also Chapter 50).

As in serum treatment, however, all the evidence suggests that the immunity acquired is predominantly type-specific; and it is clear that, with so heterogeneous a species as the pneumococcus, this factor must seriously limit the usefulness of mass immunization. It is possible that vaccination against a few prevalent types might produce a significant decrease in the incidence of the disease, even though it failed to eliminate it, or to reduce it to negligible proportions; but this we can only tell by experience.

The records of field trials on man tell a rather puzzling story. The frequency of lobar pneumonia, though very high in the aggregate, is not concentrated on particular age groups or confined in considerable part, as are diphtheria and scarlet fever, to schools and institutions. There are, however, certain environments that entail a particularly high risk of infection; and, in some of them, trials have been carried out on a considerable scale.

The three main groups of field trials that have been carried out are those by Lister (1917) among the native labourers on the Rand mines (see also Orenstein 1931); by American Army workers during the first world war (see Cecil 1925); and on Indian troops in the Baluchistan district (see Malone 1925, King 1925). In none of these trials was unequivocal evidence of protection obtained; nor was success recorded when vaccines of whole bacilli were replaced by the type-specific

but most strains produce acid, no gas, from glucose, lactose, maltose and sucrose, and coagulate milk within forty-eight hours. The organism is used in the preparation of acidophilus milk, a buttermilk with considerable acidity, for which a number of therapeutic claims have been made.⁹ Doderlein's bacillus (1892), (*Bacillus vaginalis*, *Bacillus crassus*), a common constituent of the flora of the vagina and believed to aid in the natural defenses against infection by contributing to the acidity of the vaginal secretions, is thought to be identical with *L. acidophilus*.

Lactobacillus Bifidus. Apparently closely related to *L. acidophilus* and often difficult to distinguish from it, *L. bifidus* is usually a thinner rod with ends somewhat more tapering and sometimes bifurcated (Fig. 109). It was isolated from feces of breast-fed infants by Tissier in 1900. Although common in the intestine of breast-fed infants, sometimes reaching over 90 per cent of the total intestinal flora, it is less conspicuous in the intestinal contents of bottle-fed babies. It is sometimes found also in the feces of

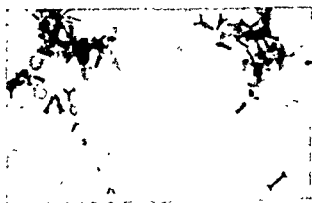


Fig. 109. *Lactobacillus bifidus*. Note the Y-shaped forms (Dack).

adult animals, including man. Antigenically it is closely related to *L. acidophilus* and like it produces acid, mainly lactic, from a number of sugars. Unlike the latter, it usually ferments inulin. It is anaerobic on primary isolation and some strains never grow well under aerobic conditions. Growth is enhanced by cystine. Partly because of its anaerobic requirements, it is sometimes classified with the Bacteroides. Tissier also isolated from feces of infants a similar organism (*Lactobacillus exilis*) which differs from the first in the possession of a more regular morphology and in greater facility of growth under aerobic conditions.

Lactobacillus Bulgaricus. This name is given to an organism isolated by Grigoroff in 1905 from Bulgarian fermented milk. It gained prominence through the work of Metchnikoff, who believed that intestinal putrefaction could be restrained by drinking milk fermented by this organism. When it was later shown that *L. bulgaricus* does not become implanted in the intestine, its use in experimental therapeutics was dropped in favor of *L. acidophilus*. More difficult to cultivate than *L. acidophilus*, slightly larger and somewhat different in sugar fermentations, it is nevertheless

⁹ For an analysis of this subject see Rettger, Levy, Weinstein and Weiss: *Lactobacillus acidophilus and Its Therapeutic Application*. Yale University Press, New Haven. 1935

Felton and Prather (1939) record that in man a *negative* reaction to an intradermal injection of a compound of specific polysaccharide with homologous antibody may be used to indicate the presence of antibody in the circulation. It remains to note that the presence in the serum of the "C-reactive" substance during the acute stage of pneumonia and other febrile diseases (see Chapter 49) is accompanied by a skin reactivity to the C-substance of the pneumococcal cell (Francis and Abernethy 1934).

PNEUMONIA IN THE LOWER ANIMALS DUE TO *H. BRONCHISEPTICUS*

In 1911 McGowan isolated *H. bronchisepticus* from the respiratory tract of a number of dogs, cats, rabbits and guinea-pigs. He regarded the organism as a primary cause of canine distemper—a view that has since been disproved (see Chapter 88). *H. bronchisepticus* is now known to be a frequent cause of infectious pneumonia in the lower animals, particularly in guinea-pigs (Smith 1913). It is present often in pure culture in the bronchi. Injected intraperitoneally into guinea-pigs in a dose of 0.5 ml. of a 24-hours' broth culture it causes death in 24 to 48 hours; *post mortem* there are small hæmorrhages on the peritoneum, and a viscid translucent exudate forming pseudomembranes on the liver, spleen, and the less mobile parts of the intestine; the bacilli are easily recovered from the peritoneal cavity, but with difficulty from the blood, liver or lungs. Subcutaneous injection produces a local lesion only; feeding and inhalation are without effect. The organism is non-pathogenic to mice. It rapidly loses its virulence in culture outside the body.

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closely related. Sherman and Hoge have reported¹⁰ that *L. bulgaricus* rarely grows at 15° C., dies out on repeated culture in a lactose-peptone-yeast extract broth, is unable to grow in media containing 2.5 per cent NaCl and fails to grow in broth at pH 7.8, while *L. acidophilus* will grow under all these conditions. The Boas-Oppler bacillus, first seen in 1895 in the gastric juice of patients suffering from carcinoma of the stomach and cultivated



Fig. 110

Fig. 111.

Fig. 110. Ground section of human tooth enamel showing an unstained bacterial plaque growing on the surface. Note the enamel rods and filamentous bacteria. $\times 1200$ (Blayney).

Fig. 111. Ground section through an early carious lesion in human tooth enamel stained with gentian violet. Note the intimate contact of the bacterial plaque with the eroding surface. $\times 500$ (Blayney).

by Heinemann and Ecker¹¹ from patients with this and other gastric diseases, is a member of this group, similar to, if not identical with *L. bulgaricus*.

DENTAL CARIES

There is much disagreement concerning the basic factors responsible for susceptibility and resistance to dental decay, and there is a wide diversity of opinion as to the relative importance of general systemic conditions, diet, bacterial flora of the mouth and various local factors in the oral environment. Nevertheless, it has been well established that certain microorganisms, particularly members of the lactobacillus genus, are usually associated with the disease and, although there is not as yet direct experimental proof, considerable indirect evidence has been adduced to indicate that this association is

¹⁰ Sherman and Hoge: Jour. Bact., 1940, 40: 11.

¹¹ Heinemann and Ecker: Jour. Bact., 1916, 1: 435.

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NON-SPORE-FORMING ANAEROBIC BACILLI (BACTEROIDES)

There is a large group of non-spore forming anaerobic bacilli that are usually gram negative. Normal inhabitants of the upper respiratory tract, genital tract and colon, where they may outnumber the aerobic flora. These microorganisms are not infrequently associated with ulcerative processes of the mucous membranes and may, under appropriate circumstances, invade the tissues and organs of the body with the production of abscesses or the blood stream to give rise to septicemia. Generally neglected in routine bacteriological examinations, these bacteria may be present in "sterile" fluids from surgically drained abscesses and similar affections where bacteria are not found by the usual cultural methods. Dack²¹ has noted their presence in 200 of 5180 specimens from the Department of Surgery of the University



Fig. 115. *Bacteroides funduliformis*. The swollen and filamentous forms and staining "ghost cells" are typical of the usual stained smear preparations. X 1000.

of Chicago submitted for routine bacteriological examination, an incidence of about 4 per cent.

The relation of these microorganisms to other bacteria is uncertain; a group they probably make up several genera and cannot be regarded as species of a single genus except tentatively. They are morphologically heterogeneous, varying from slender rod forms which may be tapered at the ends, the so-called fusiform bacilli, to filamentous and branching forms characteristic of the higher fungi. The usual appearance of stained smear preparations is illustrated in Fig. 115 and details of the cell morphology in the electron micrographs in Fig. 116. The high degree of pleomorphism characteristic of these forms has been shown by Smith, Mudd and Hillier²² to be due in large part to a mode of reproduction in which large round bodies are formed from which daughter cells separate. These usually resemble the bacillary parent cells but sometimes occur as much smaller elements in the so-called L type of division. The process by which this occurs is illustrated in the series of electron micrographs in Fig. 117 which were arranged for present purposes by Smith from Smith, Mudd and Hillier.²³

²¹ Dack: Bact. Rev., 1940, 4:227.

²² Smith, Mudd and Hillier: Jour. Bact., 1948, 56:603.

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Single species have been given a variety of generic names, including *Bacillus*, *Bacterium*, *Necrobacillus*, *Bacteroides*, *Corynebacterium*, *Streptothrix*, and *Actinomyces*. Descriptions of these bacteria have been compiled by Weinberg, Nativelle and Prevot,²⁶ and Prevot²⁷ has suggested a more or less elaborate classification. In the absence of further information neither the relation of these anaerobic forms to other bacteria nor their relation to one another can be satisfactorily formulated, and they will be discussed here under the single generic name *Bacteroides*.

Those forms which are present in great numbers in normal feces will grow upon the usual laboratory media. Those which are associated with

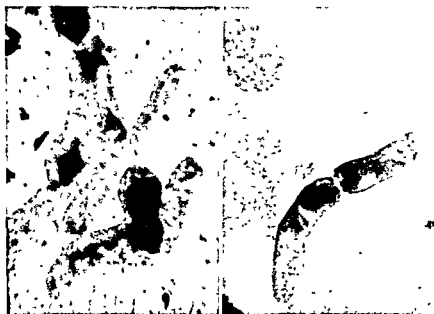


Fig. 116. *Bacteroides funduliformis*. Electron micrographs. Right, a pair of cells resulting from simple fission, fixed in formalin, $\times 3300$. Left, swollen cells containing granular material, especially in the swollen areas, fixed in formalin, $\times 4900$. (Smith, Mudd and Hillier, Jour. Bact., 1948, 56:603.)

pathological processes in man, however, are nutritively fastidious and require infusion media enriched by the addition of blood, yeast or vegetable extracts and similar substances, together with glucose and cysteine. In some cases they may be isolated on beef or veal infusion blood agar. Some strains grow in amino acid media supplemented with all the known bacterial vitamins, pyruvic acid appears to be of considerable importance in the nutrition of these organisms. The optimum pH is 6.3 to 7.0 and the optimum temperature for growth is 37° C. Completely anaerobic conditions are essential and growth is favored by the presence of carbon dioxide.

The better known of these bacteria may be described briefly.

Bacteroides Fusiformis (*Bacillus fusiformis*, *Fusiformis fusiformis*, *Fusobacterium plauti-vincenti*). These fusiform bacilli are found in ulcerative

²⁶ Weinberg, Nativelle and Prevot: *Les Microbes Anaerobies*. Masson et Cie, Paris, 1937.

²⁷ Prevot: *Manuel de Classification et de Determination des Bacteries Anaerobies*. Monographies de l'Institut Pasteur, Masson et Cie., Paris, 1940.

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stomatitis (trench mouth) and Vincent's angina. Their relation to the spirochetes with which they are usually associated is considered elsewhere (p. 735). These forms are regarded by Bergey as the type species of the genus *Fusobacterium*. They appear as slender rectilinear or incurving bacilli and frequently assume a filamentous form. They are gram-negative and tend to stain irregularly with the ordinary aniline dyes. Acid but no gas is produced from dextrose, levulose, sucrose, maltose, and sometimes from lactose. They may be isolated on blood agar incubated anaerobically, and the colonies are small and surrounded by a zone of green hemolysis. *Bacteroides fusiformis* is not pathogenic for experimental animals in pure culture, but in mixed culture produces abscesses.

Bacteroides Fragilis (*Bacillus fragilis*, *Fusiformis fragilis*). This bacterium was found by Veillon and Zuber in twenty-two cases of appendicitis and since has been found in lung, pelvic and hepatic abscesses, in septicemias with metastatic abscesses, and in infections of the urinary tract. The cells are small, slender rods, sometimes slightly curved. They are gram-negative and non-motile. *Bacteroides fragilis* is difficult to isolate but will grow on the usual laboratory media. The colonies are small (less than 1 mm.) and transparent. No hemolysis is apparent on blood agar. There is a marked tendency to autolysis with apparent resorption of the colonies and broth cultures are no longer viable after seven to eight days' incubation. A variety of sugars are fermented to acid and gas. The pathogenicity of this bacterium for experimental animals is uncertain.

Bacteroides Funduliformis (*Actinomyces necrophorus*, *Fusiformis necrophorus*, *Streptothrix necrophorus*, *Bacillus necrophorus*, *Corynebacterium necrophorum*, Schmorl's bacillus). These bacilli have been found in abscesses of the liver, lung and other parts of the body, in chronic ulcerative colitis and in the blood stream. Infections with these microorganisms are probably more common than is generally realized. *Bacteroides funduliformis* has also been found in lower animals as in bovine liver abscesses. The bacilli are highly pleomorphic, slender straight and curved rods may be found intermingled with filamentous and swollen forms, and "ghost cells" which do not stain are frequent (Fig. 115). There is a marked tendency to irregular staining and the bacilli are gram-negative. The colonies on blood agar are variable in size from plate to plate and surrounded by a zone of green hemolysis which may become clear upon prolonged exposure to the air. Glucose, maltose and levulose are fermented to acid, and there is no evidence of proteolytic activity in gelatin or coagulated egg-white cultures. Some strains give rise to a spreading necrotic lesion upon subcutaneous injection in the rabbit which is usually fatal, while others produce only a localized lesion. Guinea pigs are relatively resistant.

Bacteroides Ramosum (*Fusiformis ramosus*, *Ranubacterium ramosum*, *Bacillus ramosus*. Not to be confused with *Bacillus ramosus*, a name sometimes applied to *Bacillus mycoides*). These bacteria are frequently present in the pus of appendicitis and have been encountered in pulmonary gangrene.²⁸ They appear as small, slender rods which often show branching forms and pseudofilaments. They are gram-positive. *Bacteroides ramosum*

²⁸ Cf. Jour. Amer. Med. Assn., 1937, 108:1902.

if used in reference to non-febrile infections with *Brucella* organisms, which are now being recognized as increasingly common. Hence the term Brucellosis has been suggested on analogy with tuberculosis, to apply to all types of *Brucella* infections, febrile or non-febrile, overt or latent, in animals or in man. This term has little to recommend it, since these considerations apply almost equally to diseases caused by other infective agents. We prefer ourselves to use instead the term "*Brucella* infection."

For the sake of clarity we propose to give a separate description of the epidemiology of undulant fever in man due to the three type organisms. We shall deal first with infections caused by *Br. melitensis*, including in our description the general symptomatology and bacteriology of the disease.

Undulant Fever due to *Br. melitensis*

Synonyms: Malta fever, Mediterranean fever, Neapolitan fever, Country fever of Constantinople, New fever of Crete, Rock fever of Gibraltar. French: *Mélioococcie*.

Quoting from the classical monograph of Hughes (1897): "Clinically, the fever has a peculiarly irregular temperature curve, consisting of intermittent waves or undulations of pyrexia, of a distinctly remittent character. These pyrexial waves or undulations last, as a rule, from 1 to 3 weeks, with an apyrexial interval, or period of temporary abatement of pyrexial intensity between, lasting for 2 or more days. . . . This pyrexia is usually accompanied by obstinate constipation, progressive anæmia, and debility. It is often complicated with, and followed by, neuralgic symptoms referred to the peripheral or central nervous system; arthritic effusions; painful inflammatory conditions of certain fibrous structures, of a localized nature; or swelling of the testicles." Numerous clinical types are recognized, such as the malignant, the undulatory, the intermittent, and the irregular type. In addition, there are subclinical infections, which can be diagnosed only by bacteriological methods, and infections that may remain latent for weeks or months before flaring up or retrogressing. For a description of these the reader is referred to Marston (1863), who first separated Malta fever from other fevers, to Hughes (1897), whose clinical study has never been improved upon, and more recently to Rainsford (1935) and Lisbonne and Janbon (1935). Several complications may occur, beyond those already mentioned, including osteomyelitis, which usually take the form of localized bone abscesses, bronchitis, and acute inflammation of one or other of the visceral organs (see Michel-Béchet *et al.* 1939).

The commonest symptoms are probably asthenia, fever, muscular and articular pains, nocturnal sweats—often drenching—anorexia, constipation, nervous irritability, and chills or rigors (Taylor and Hazemann 1932).

The incubation period is variable. It may be as short as a week or as long as several months. Most commonly it is 10-30 days. The duration of the illness varies from a few days to over a year, 3 months being the usual time.

Bacteriology.

Bacteriologically, the work of Bruce (1887, 1888, 1893), of Hughes (1893, 1897), and of the Mediterranean Fever Commission (Report 1905-07) showed that the disease was a septicæmia. Working in Malta, Bruce (1893) was able on two occasions to grow the organism from the juice obtained by splenic puncture, and subse-

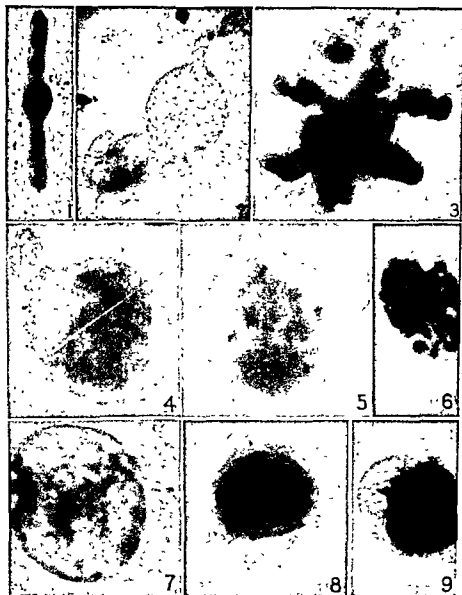


Fig 117. Reproduction by large bodies in *Bacteroides funduliformis*. In liquid media the cells develop rounded enlargements (1) which increase in size (2) to yield large round bodies (4-9). These germinate by extrusion of multiple filaments (3). Note that the filaments are sheathed with the cell wall of the large body. The intracellular dark material breaks off with the filaments which then are used by daughter cells (4 and 5), (6 and 7), (8 and 9) to form new cells. (Rear-

neither water, milk, nor any other article of food seemed to be responsible in any way; he favoured a theory of contagion, either direct or through the agency of mosquitoes.

It was left to Zammit of the Malta Board of Health, and a member of the Commission on Mediterranean Fever, to indicate the true path of infection. In his first communication (1905) he reported that 5 out of 6 goats which he had examined gave a positive agglutination reaction to *Br. melitensis*. From the blood of two of these animals, he succeeded in cultivating the actual organism itself. Horrocks (1905) rapidly confirmed this, and further demonstrated the frequent presence of *Br. melitensis* in the milk and the urine of apparently healthy goats. Kennedy (1905), who examined the blood serum of 161 goats from 8 different herds, demonstrated the presence of specific agglutinins in no fewer than 84 or 52.2 per cent. of specimens. This high incidence of infection was found to exist not only in the public herds, but in those animals that were kept privately under special care.

As it was evident that goats' milk was probably the main source of infection,

TABLE 150
UNDULANT FEVER IN MALTA
(Taken from Eyre 1908, 1912).

	Civil.		Navy.		Army.	
	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
1901 . . .	642	54	252	3	253	0
1902 . . .	624	45	354	2	155	6
1903 . . .	589	48	339	6	404	9
1904 . . .	573	59	333	8	320	12
1905 . . .	663	88	270	7	647	16
1906 . . .	822	117	145	4	163	2
1907 . . .	714	78	12	0	9	1
1908 . . .	502	?	6	—	5	?
1909 . . .	456	?	10	—	1	?
1910 . . .	318	?	3	—	1	?

it was desirable to ascertain on a large scale the proportion of infected animals. For this purpose, Zammit's test was found to be of great value. It consists in observing the presence of agglutinins, not in the blood serum, but in the milk of the goats. The proportion of infected goats judged by this test is much lower than that given by the serum agglutination test, since only 60-85 per cent. of the animals showing blood agglutinins also contain agglutinins in their milk. Zammit (1906) examined 710 samples of milk, and obtained 133 positive reactions. From every milk giving a strong reaction he was able to isolate *Br. melitensis* in culture. Horrocks and Kennedy (1906), who made numerous investigations both by the serum and by the milk agglutination test, came to the conclusion that 41 per cent. of the goats in Malta were infected, and that 10 per cent. of those that supplied milk were excreting *Br. melitensis*. It was found difficult to recognize the infected animals on clinical grounds; they did not suffer from a true fever, and though some showed loss of weight, a thinning of the coat, and a short hacking cough, others remained perfectly well and displayed no sign of illness.

is pathogenic for experimental animals, giving rise to abscesses in rabbits and guinea pigs on subcutaneous injection; fatal infections are produced by intravenous inoculation.

Bacteroides Melaninogenicum (*Ristella melaninogenica*). This microorganism has been found in the mouth, tonsils, infected abdominal wounds, focal infections of the kidneys, in stools from patients with chronic amebic dysentery, and in puerperal sepsis. It is described by Burdon²⁹ as a very small gram-negative diplobacillus. On blood agar its colonies are black owing to the slow (four to five days) formation of a melanin-like pigment. *Bact. melaninogenicum* grows well in mixed culture, but sparsely in pure culture. It is difficult to obtain in pure culture and, when admixed with other bacteria in a colony, colors the entire colony black. Acid is formed from dextrose, levulose, lactose, maltose, sucrose and mannitol. It is markedly proteolytic and rapidly digests coagulated serum and other native proteins. Its pathogenicity as a primary invader is uncertain.

Bacteroides Pneumosintes (*Dialister pneumosintes*). *Bacteroides pneumosintes* was described by Olitsky and Gates³⁰ as the causal agent of influenza. Now known to bear no relation to this disease, its pathogenicity for man is uncertain. It may be cultured from nasopharyngeal washings in Smith-Noguchi medium (human ascitic fluid containing a piece of sterile rabbit kidney, and covered with a petrolatum seal) and after a few transfers will grow anaerobically on blood agar, chocolate agar and Boder's medium. These bacteria are minute bodies arranged singly, in pairs, or in short chains; they are non-motile and gram-negative. Because of their small size they pass Berkefeld V and N filters. They produce acid but no gas from dextrose; other sugars are not fermented. If mass cultures are injected intratracheally into rabbits there is a rise in temperature and sometimes a conjunctivitis and mononuclear leucopenia with recovery in two or three days. If the animal is sacrificed the lungs are found to be edematous with hemorrhages on the surfaces. *Bact. pneumosintes* is not pathogenic for monkeys.

BARTONELLA

Bartonella Bacilliformis. Oroya fever, an infectious anemia, and veruga peruana, a disease characterized by miliary or nodular eruptions, have existed for centuries in certain districts in Peru, and recently have been found in Colombia and Ecuador. It was shown by Carrion, through fatal self-inoculation, that the two are stages or manifestations of a single disease which is now commonly known as Carrion's disease. The etiologic agent is a small pleomorphic bacillus which was observed by Barton in 1905 and named *Bartonella bacilliformis* by Strong, Tyzzer and Sellards.³¹

Morphology and Staining. *Bartonella bacilliformis* is a small, motile, aerobic, gram-negative bacillus 0.2 to 0.5 μ in diameter and 1 to 2 μ in length which is found as a slightly curved rod occurring singly, end to end in pairs, and in short chains. A rounded ovoid form, 0.3 to 1 μ in diameter.

²⁹ Burdon. Jour. Inf. Dis., 1928, 42:161.

³⁰ Olitsky and Gates Jour. Exp. Med., 1921, 33:125, 361, 713, *ibid.*, 1922, 35:813, *ibid.*, 1922, 36:501.

³¹ Strong, Tyzzer and Sellards: Jour. Amer. Med. Assn., 1915, 64:806

virus had been recognized. It must of course be admitted that proof of the way in which infection spreads may not become possible till the identity of the parasite is known; but apart from immunization of the host, measures for preventing infectious disease are as a rule most profitably directed to control or eradication of the reservoir of infection or to interference with the means by which infection is transmitted to the susceptible host.

The second point of interest is that, in an epidemiological inquiry, it is harder to assess the importance of a given factor when it operates more or less uniformly throughout a population than when it is distributed in such a way as to affect different sections unevenly. Both the epidemiologists who investigated the disease on Malta (Johnstone 1905, Davies 1906) reported that there was no evidence to suggest that infection was conveyed by food. Both of them overlooked the fact that a disease spread by an article of almost universal consumption, such as goats' milk, would show little preference for any one class of the population, if the contamination of the milk were widespread and frequent rather than local and occasional. The clue to the problem was furnished by the bacteriologist. It was this second discovery, that a large proportion of goats contained agglutinins in their blood for *Br. melitensis*, which complemented the first discovery, that of the organism itself, and pointed out the way by which the spread of the disease might be checked.

(For an excellent account of the epidemiology of Malta fever in the Royal Navy, see Dudley 1931.)

Epidemiology in Other Countries.—The next most important bacteriological and epidemiological study of the disease has been made by Taylor and his colleagues in the South of France, working at the Undulant Fever Centre established at Montpellier by the Rockefeller Foundation (see Taylor and Hazemann 1932, Taylor, Lisbonne, and Roman 1932, Taylor, Lisbonne, and Vidal 1935, Taylor *et al.* 1938a). The disease was found to be widespread in the south-east of France, where sheep and goats are common and cattle are relatively few. The incidence in males was 2-3 times that in females, and was highest in the 15-45 age group. The occupational incidence was very striking, the majority of the cases occurring in the agricultural community, especially among those engaged in rearing sheep and goats. The seasonal prevalence was at its maximum in the spring, corresponding to the time of lambing and abortion. Though the consumption of goats' milk, and of fresh cheese made from goats' or ewes' milk, undoubtedly played some part in infection (see Carrieu and Lafenêtre 1932, Veloppé and Jaubert 1935), the evidence on the whole suggested that infection most frequently resulted from direct contact with infected animals and manure. In a population, however, living under very primitive conditions, and exposed to many different sources of infection, it was difficult to ascertain precisely the most important route by which the organisms gained access to the body. The very interesting observation was made (Taylor, Vidal and Roman 1934) that cows in close contact with sheep and goats might become infected with *Br. melitensis*, excrete this organism for months in the milk, and give rise to undulant fever in human beings consuming it in the raw condition.

The disease affects several other countries, including Italy, Spain, Greece, Transcaucasia, Algeria, Tunis, Palestine, Arabia, India, South Africa, China, and various parts of South America. Like Malta, Corsica and Sicily are seriously affected. In this country, owing to the absence of *melitensis* infection in sheep

is also observed singly, in pairs and in groups. It stains reddish violet with Giemsa's stain, sometimes showing a reddish purple granule at one end of a bluish rod.

Physiology. This microorganism was first cultivated in semisolid leptospira medium and later in tissue culture and in the developing chick embryo, though it could not be carried in serial transfer in the last. Sparse growth occurs on cystine-dextrose-blood agar and apparently the X factor is required while the V factor is not. Gieman³² has developed a tryptone-serum medium in both liquid and solid form which supports excellent growth. Little is known of its physiological processes.

Pathogenicity for Man. As indicated above, the disease occurs in two forms, the systemic form in which the red cells are infected, and the histoid cutaneous

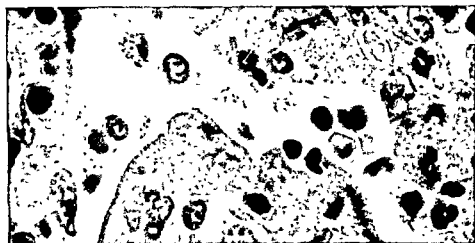


Fig. 118. *Bartonella bacilliformis* in human spleen. Note the huge numbers of the microorganisms packed into the lining cells. Giemsa, $\times 1450$ (Humphreys).

form. The two may exist independently, coexist or occur separately, though the usual course is the systemic form followed by the cutaneous form if the former is not fatal, or the cutaneous form alone. The first is a severe, often fatal, febrile anemia. The incubation period is given as about three weeks. The anemia is frequently severe, and the loss of red cells may be 200,000 to 300,000 per cubic millimeter per day until the total count is half a million or less. The anemia is thought to be due to the direct action of the microorganisms on the erythrocytes, including hemolysis, and to tissue hemorrhages. The fever is of an irregular remittent type, and pain in the bones, joints and head is common. The case fatality rate is 20 to 40 per cent, death occurring in two to three weeks after onset. With recovery the eruptive stage of the disease appears and persists for two to three months. Whether it follows the systemic form or is the primary clinical manifestation of infection, this stage is characterized by a miliary or nodular eruption; the former is by far the more common. The miliary eruption is most common on the face and extremities, appearing as a macule which becomes nodular and eventually disappears leaving no scar. The nodular type of eruption develops more slowly, the nodules may become 2 to

³² Gieman: Proc. Soc. Exp. Biol. Med., 1941, 47-329.

and Grilichess 1932, Messer 1932, Dettling 1932, Wade 1933, Hall 1933, Russ 1934, Kalbfleisch and Kalbfleisch 1935.) It will be seen that the incidence of the disease, calculated per million of the population, is much the same in Great Britain, Germany, Austria, Holland, and Sweden, but that it is considerably higher in Switzerland and Denmark. In the United States undulant fever is known to be very prevalent, but as some of the cases are due to infection with *Br. suis*, and the antigenic structure of this organism is closely related to that of *Br. abortus* (see Chapter 34), figures based on agglutination results are not strictly comparable with those just given. There is evidence to suggest that the different incidence in different countries is dependent to some extent on the frequency of contagious abortion in cattle, and the degree of exposure of the human population, though other factors undoubtedly play a part (Henricsson 1932, Olin 1935). Some of the difference is almost certainly more apparent than real, and is due to the much better diagnostic facilities available in certain countries than in others. Most cases of undulant fever are sporadic, but occasional small outbreaks occur (Elkington *et al* 1940, Steele and Hastings 1948,

TABLE 152

PROPORTION OF WIDAL SERA AGGLUTINATING *Br. abortus* TO 1/80 OR OVER, AND ESTIMATED ANNUAL NUMBER OF UNDULANT FEVER CASES ABOUT 1930.

(Figures compiled from several papers in different countries; for references, see text.)

Country.	No. of Sera Examined	No Positive	Percentage Positive.	Estimated Annual Number of Undulant Fever Cases	Estimated No. of Cases per 1,000,000 Population
Great Britain	3,175	101	3.18	440	11
Germany	9,397	323	3.44	600	10
Austria	9,693	177	1.83	50	8
Switzerland	1,503	91	6.1	340	84
Holland	4,500	50	1.11	90	12
Denmark	4,623	500	10.82	500	147
Sweden	—	—	—	120	20

Anderson 1950). Further studies on the incidence of the disease may be consulted for Great Britain (Menton 1937, Soltys 1946, Dalrymple-Champneys 1950), Yugoslavia (Simitch and Djourichitch 1936), South Africa (Campbell and Greenfield 1937), Canada (Dolman and Hudson 1938), United States of America (Jordan and Bort 1946, Magoffin *et al* 1949), and the Argentine (Molinelli *et al* 1948).

Latent Infections.—Besides definite clinical disease, *Br. abortus* gives rise to a considerable amount of latent infection. Examination of Wassermann sera in this country and in Germany shows that about 1.5 per cent. agglutinate *Br. abortus* to a titre of 1/40 or over. The titre is generally less than 1/160, and high titres, such as those yielded by the majority of sera from patients with undulant fever, are uncommon. A certain proportion of these sera are derived from patients actually suffering from the disease. The majority, however, come from persons with no clinical evidence of undulant fever. The probability is that these agglutinins are due to latent infections with *Br. abortus*. This interpretation is supported by two facts. The first is that agglutinins, even in a titre of 1/20, are rarely present in the sera of persons who do not drink raw milk or cream, and who are not exposed to contact with infected animals. The second is that agglutinins are

3 cm. in diameter and have a tendency to become strangulated. They are formed by the proliferation of the endothelial cells of the vessels which become obstructed with an inflammatory exudate of plasma cells and fibroblasts, and show a marked tendency to hemorrhage.

Bartonella cells are found in large numbers within the erythrocytes in Oroya fever and may be demonstrated in Giemsa-stained blood smears. In both forms of the disease they are found in the tissue macrophages, especially the vascular endothelial cells of the lymphatics, spleen and liver, often in large clusters within individual cells. It is reported that blood cultures are frequently positive in the systemic infection but it is not clear whether this is a reliable method of diagnosis.

Epidemiology. Bartonellosis is strictly limited geographically, being, so far as is known, exclusively American and tropical, occurring in the Andes between latitudes 2° N. and 13° S. It is transmitted by *Phlebotomus verrucarum* and *P. noguchii* in Peru; whether other species of *Phlebotomus* or other arthropods, such as *Dermacentor*, are natural vectors is not known. The reservoir of infection, other than asymptomatic infections and clinical disease in man, is not known but domestic animals, including chickens, and guinea pigs and field mice are suspected.³³

Immunity. It is generally said that recovery from an attack of either form of the disease confers a solid immunity to both. With the cultivation of *B. bacilliformis*, it has been possible to study the occurrence of circulating antibody, agglutinins, and investigate the possibilities of prophylactic inoculation. Howe³⁴ has shown that agglutinins may be demonstrated during the early stages of the disease but, in spite of the lasting immunity, almost always disappear with the subsidence of clinical symptoms. Their diagnostic significance is not as yet known. Howe and Hertig³⁵ have carried out a limited series of inoculations with formalized suspensions of *B. bacilliformis* with encouraging results.

Pathogenicity for Animals. In general experimental animals appear to be highly resistant to infection with *B. bacilliformis*. Both the eruptive and systemic forms of the disease have been produced in rhesus monkeys, the latter in splenectomized animals.

Bartonellosis of Animals. Naturally occurring bartonellosis has been found in a number of animals, including the dog, cattle and a variety of rodents. The infection takes a systemic rather than eruptive form and is usually latent but activated when host resistance is reduced as by splenectomy. The bartonella-like organisms of lower animals have been separated into three genera, *Haemobartonella*, *Grahamella* containing but a single species (*Grahamella talpae*) which differs from *Haemobartonella* in that the infection is not eradicated by treatment with arsenicals, and *Eperythrozoon*, which is more highly pleomorphic than the first two groups and is found in the plasma as well as in the erythrocytes. None of these forms have been cultivated and all appear to be non-pathogenic for man.

³³ For an excellent review of the present status of human bartonellosis see Mera: Bol. Oficina Sanitaria Panamericana, 1943, 22:304.

³⁴ Howe Jour. Exp. Med., 1942, 75:65, Arch. Int. Med., 1943, 72:147.

³⁵ Howe and Hertig Jour. Immunol., 1943, 47:471

to mild infection more or less continuously. Garrod (1937) drew attention to the susceptibility of *Br. abortus* to the bactericidal action of the gastric juice, and suggested that this might be one reason why undulant fever is not commoner than it is. It would be idle to pretend, however, that we have any exact knowledge of the factors determining the course of infection in any given individual, and those who deny the pathogenicity of *Br. abortus* for man, because not every exposed person develops the disease, must be wholly unaware of the complexity of the equilibrium existing between host and parasite. The possibility that strains of *Br. abortus* vary in virulence must be considered, but so far there is no evidence to support it (see Birch and Gilman 1935, Olin 1935).

Mode of Infection.—The mode of infection varies in different countries and in different parts of the same country. The main source is obviously cattle, and man becomes infected by consumption of raw milk or cream, or by contact with infected animals, either alive or dead. Generally speaking, the town population is exposed to infection from milk, whereas the country population is exposed to both milk-borne and direct contact infection.

Numerous records in different countries show that a considerable proportion of samples of raw milk coming from infected herds contain living *abortus* bacilli. In Great Britain about 20–30 per cent. of herd samples are infected. Experience has shown that *consumption of raw infected milk* is attended by a certain risk of contracting the disease, whereas consumption of the same milk after pasteurization or other effective heat treatment is harmless. In London, where about 98 per cent. of the milk is heat-treated, and in the large cities of the United States where most of the milk is pasteurized, undulant fever is practically unknown. The cases that do occur are mostly among those who drink Tuberculin Tested or other types of raw milk, either at home or on a visit to the country.

Several careful studies made in institutions and small communities supplied with infected raw milk have shown that a considerable proportion of the population develop serum agglutinins, that a small proportion suffer from sub-clinical and clinical forms of undulant fever, while, in a larger proportion, the infection remains completely latent. Removal of the infected animals, or pasteurization of the milk supply, is followed by cessation of clinical illness and by a gradual disappearance of agglutinins from the sera of latently infected persons (see King and Caldwell 1929, Hasseltine and Knight 1931, Sasano, Caldwell and Medlar 1931, Dooley 1932, Johns, Campbell and Tennant 1932, Wilcox 1932, Carpenter and Boak 1933, Welch and Mickle 1933, Hall and Learmonth 1934, Cameron and Wells 1934, Stone and Bogen 1935, von Engel 1938, Elkington *et al.* 1940, Cruickshank and Stevenson 1942).

It may be pointed out here that alimentary infection is almost always due to the consumption of raw milk or cream. *Butter and cheese*, except when made from untreated milk and consumed within a week or two, are probably inoffensive, since the organisms die out very rapidly as the result of lactic fermentation (Mazé and Césari 1931, Caronna

is on record (Huddleson and Munger 1940).

Contact infections are mainly occupational, occurring in towns among slaughterers and meat packers, and in the country among the farming population. Since

Of these, the most commonly encountered is *Haemobartonella muris* which infects rats and is transmitted by the rat louse, *Haematopinus*, and the rat flea, *Xenopsylla cheopsis*. The infection is very common and the great majority of laboratory rats are infected. The infection is latent, and may be precipitated by splenectomy in an acute form with the appearance of parasitized red cells in the circulating blood. Special strains of rats are maintained bartonella-free for experimental purposes. *Haemobartonella canis* is the cause of an infectious anemia of dogs and is transmitted by the dog flea, *Ctenocephalus*. *Haemobartonella tyzzeri* occurs in guinea pigs, *Haemobartonella microtii* in the vole, *Haemobartonella bovis* in cattle, and other species in mice, shrews and squirrels.

Eperythrozoonosis similarly occurs as a latent infection which is activated by splenectomy. *Eperythrozoon coccoides* is a parasite of white mice and other species occur in wild mice. The disease also occurs in sheep and cattle and the causative organisms are *Eperythrozoon ovis* and *Eperythrozoon wenyonii* respectively.

The Systematic Position of Bartonella. The relationship of these microorganisms to the bacteria on the one hand and to the rickettsiae and viruses on the other is of some interest. Among the bacteria the tendency to parasitize the cells of the host and appear as intracellular clumps of microorganisms is most marked in *Pasteurella tularensis* which commonly is found in an intracellular position. Bartonella shows a much more marked preference for intracellular parasitism and both in this respect and in morphology seems to be closely related to the rickettsiae. It is generally agreed, however, that the relationship is not sufficiently close to justify their classification as rickettsiae and they may be considered as lying between them and the bacteria.

THE PLEUROPNEUMONIA-LIKE ORGANISMS¹⁴

The first of an apparently more or less homogeneous group of highly pleomorphic, filterable microorganisms to be described is the causative agent of *bovine pleuropneumonia*. Since its first study in 1898 a number of similar forms, both parasitic and saprophytic, have been found. For want of a better name they have been called the pleuropneumonia group or pleuropneumonia-like organisms, though none of the rest produces pleuropneumonia in cattle. Their relationship to other microorganisms is almost completely unknown and they have been grouped with the viruses by some because of their filterability, with the rickettsiae by others because of a predilection for an intracellular existence in the host, and, as indicated earlier, by still others they are grouped with the actinomycetes.

Morphology and Staining. There are probably few if any other microorganisms which are as highly pleomorphic as these forms. Impression preparations of colonies show granules of various sizes, filaments which may be branched and contain streaming protoplasm, balloon and disc like structures, ring, club and star forms, and ameboid structures. Filaments are more numerous in recently isolated cultures and tend not to be formed in older stock cultures. The microscopic morphology of these forms is illustrated in a Giemsa-stained

¹⁴ These microorganisms are discussed in detail in the review by Sabin. *Bact. Rev.*, 1941, 5:1.

Adult males are almost solely affected (Hasseltine 1930). Occasionally *Br. suis* infects cattle, and milk-borne outbreaks of undulant fever, without any special occupational incidence, are likely to occur (see Beattie and Rice 1934, Horning 1935, Borts *et al.* 1943). *Brucella* infection of pigs is far less common than contagious abortion of cattle. In consequence undulant fever due to *Br. suis* has hitherto been restricted mainly to the middle-west of North America, to Brazil, and to the Argentine (Molinelli *et al.* 1918).

Diagnosis of Undulant Fever

The occurrence of subclinical and atypical infections renders the clinical diagnosis of undulant fever peculiarly difficult, and all observers are agreed on the fact that large numbers of cases are missed. The final diagnosis can be made only by bacteriological examination. In practice it is well to regard every case of pyrexia of undiagnosed origin as possibly due to *Brucella* infection, and to bear this disease in mind during the investigation of acute and chronic inflammatory conditions in which the diagnosis remains doubtful. Help is sometimes afforded by a blood count, which usually shows a secondary anaemia, a mild leucopenia, and a relative lymphocytosis.

Blood Culture.—In the febrile stages of the disease, an attempt should be made to isolate the causative organism from the blood stream.

The patient's blood should be withdrawn while the temperature is elevated, and preferably during the rise of a pyrexial wave. It should be distributed in 3-5 ml. quantities into two flasks containing 20-100 ml. of glucose broth, serum broth, Bacto tryptose broth, or liver extract broth. One of the flasks should be incubated aerobically, the other in an atmosphere in which 10-25 per cent. of the air has been replaced by CO₂. Subcultures should be made every 3-5 days on to a solid medium, and these should be incubated in the same atmosphere as the parent culture. Growth is often slow, and no culture should be discarded in less than 4-8 weeks. To guard against contamination, 1/20,000 Victoria blue (Hauptmann 1937), or 1/700,000 crystal violet (Huddleson 1939), may be added to the culture. Two ml. of the patient's blood may be inoculated intraperitoneally into guinea-pigs (Poston 1938). If fluid blood is not available, blood clot may be ground up and cultured in the usual way (West and Borman 1945). (For other methods of culturing blood, see Elkeles and Fried 1932, Rainsford 1933, Hauptmann 1935a, Stewart *et al.* 1935, McCullough 1949, Hosty and Johnson 1953.)

Though blood culture is positive in a high proportion of *melitensis* and *suis* infections, not more than 10-20 per cent. of *abortus* infections prove positive by this method (but see Hosty and Johnson 1953). In *melitensis* infections the organism can often be isolated from the urine, provided repeated samples are examined (Kennedy 1905). Occasionally *Br. abortus* can be demonstrated in the bile (Amoss and Poston 1930, Leavell and Amoss 1931), stools (Smith 1932b, Beattie *et al.* 1935, Goldstein *et al.* 1936) and tonsils (Carpenter and Boak 1932, Poelma and Pickens 1932).

Agglutination Test.—The most generally useful method is the agglutination test, first introduced by Wright and Smith in 1897.

Final dilutions of the serum should be put up ranging from 1/20 to 1/5120, using as agglutinating suspensions strains corresponding to the prevalent type of infection in the country. Only strains that are absolutely smooth, as judged by the thermoagglutination and acriflavine tests, should be chosen. The organisms should be grown on liver extract

smear in Fig. 119 and the ring forms are well shown in the electron micrographs in Fig. 120. The marked pleomorphism is due in part to the fragility of the organisms, many of which are torn apart in making smears, and, as in the case of *Bacteroides funduliformis*, in part a consequence of modes of reproduction other than binary fission, e.g., that of the development of round bodies which become nodular with outgrowths that segment into daughter cells. This process has been studied in detail by means of electron micrographs by Smith, Hillier and Mudd³⁷ and is illustrated in Fig. 122. Viable structures vary greatly in size and include ultramicroscopic elements which are filterable. Edward³⁸ describes experiments on filtration through gradocol membranes (p. 844) in which the concentration of organisms in an emulsion was reduced from 10^8 to 10^5 by passage through a membrane of APD 0.8μ . This titer decreased

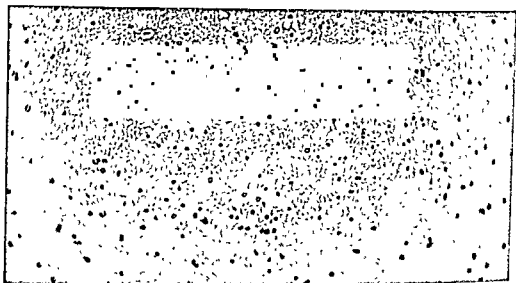


Fig. 119. Two-day culture of Type A pleuropneumonia-like organism from mice in serum-dextrose broth. Note the rings and elementary bodies. Giemsa; $\times 1000$ (Sabin, Bact. Rev.).

progressively with membranes of decreasing pore size but complete retention did not occur until a membrane of 0.33μ was used. In contrast to this, the end point is usually sharp in the filtration of viruses. On the basis of these observations the diameter of the smallest viable elements was estimated to be 165 to $247 m\mu$. Similar estimates vary somewhat for other strains, the agent of pleuropneumonia, for example, is 125 to $175 m\mu$.

These forms cannot be found in tissues by any method of staining. They are not demonstrable in smears stained by the usual aniline dyes, but are stained by certain polychrome stains, Giemsa and Castaneda's rickettsia stain. Very thick films prepared from the sediment of centrifuged cultures are gram-negative.

On primary isolation or change to a slightly different culture medium, broth cultures sometimes, but not always, show no detectable evidence of growth and may be carried along by "blind passage" for several transfers before it appears. Some strains show a uniform opalescence, others a granular type of

³⁷ Smith, Hillier and Mudd: Jour. Bact., 1948, 56:589

³⁸ Edward. Jour. Path. Bact., 1940, 50:409.

evidence for this statement is not very satisfactory. Apart from the anamnestic reaction, which is not likely to account for more than a slight raising of the titre (see Amoia 1933), the most common cause of non-specific agglutination is the use of a suspension made from strains that are not absolutely smooth. Such strains are often agglutinated by normal serum, and apparently even more often by the sera of febrile patients. Their use in the past has been very common and has given rise to much confusion in the literature. On the other hand, it is stated that the serum of persons who have been inoculated against cholera may agglutinate *Brucella* to a titre as high as 1/160 (Eisele *et al.* 1917). With this possible exception it is generally permissible to conclude that an elevated titre to a smooth suspension is indicative of infection—latent, active or past—with an organism of the *Brucella* group.

The failure of serum from some undulant fever patients to agglutinate *Brucella* has been ascribed by various workers to the occurrence of non-agglutinating or so-called agglutinin-blocking antibodies (Griffitts 1947, Wilson and Merrifield 1951, Schuhardt *et al.* 1951). The blocking property is said to be destroyed by heating the serum to 56° C for 30 minutes; and sera containing the blocking antibodies may show agglutination if the test is carried out by the slide method or if the organisms are suspended in whole rabbit serum instead of in saline. Alternatively the presence of specific antibodies may be revealed by making use of the technique described by Coombs, Mourant and Race (1945), in which the organisms after exposure to the patient's serum are centrifuged, washed, resuspended in saline and treated with the serum of a rabbit immunized against human globulin. How important in diagnosis these blocking antibodies are, it is difficult to say. Most workers of experience will agree that, if the ordinary agglutination test is carried out carefully, and repeated once or twice if negative, failure to demonstrate agglutinins in patients suffering from undulant fever is uncommon (see Magoffin *et al.* 1949, Spink and Anderson 1950).

We may add that the drinking of milk containing antibodies is not followed in man by the appearance of agglutinins in the blood (see Boak and Carpenter 1929, Peterson 1935), nor is the ingestion of dead *Brucella* likely to give rise to agglutinins in normal persons, except perhaps in large doses (McCullough *et al.* 1949, Braude *et al.* 1949).

(For references to interpretation of the agglutinin test, see Bayne-Jones 1930, Wilson 1930, 1934, Ranque and Senéz 1932, Magliulo 1933, Morellini 1933, Sanfilippo 1933, Mohr 1935, Hauptmann 1935b.)

The type of infecting organism may often be determined by use of the quantitative agglutinin absorption test, described in Chapter 34, carried out on the patient's serum (see Habs and Sievert 1935).

The complement-fixation reaction may be used to confirm the agglutination test, or in suggestive cases when this test is negative. On the whole, complement-fixing bodies tend to be more species-specific than agglutinins, to appear later and to persist longer (see Sasano *et al.* 1931, Morales-Otero and Monge 1932, Laun and Heide 1931, Ithurrat *et al.* 1948). A precipitation reaction has been described (Schlesmann 1932).

Brucellin Test.—In 1922 Burnet drew attention to an allergic skin test, performed by the intradermal inoculation into the arm of 0.05–0.1 ml. of a filtrate of a 20-day-old broth culture of *Br. melitensis* (melitin) or *Br. abortus* (abortin). A positive reaction is characterized by the appearance in 6 hours of a slightly raised, sometimes tender, oedematous plaque, 2–6 cm. in diameter, distinguished in colour from the surrounding skin. Some workers insist, for a positive reaction, on a minimum diameter of 0.5 cm. induration and oedema at the end of 48 hours. A central nodule may develop and persist for several days. Pseudo-reactions generally appear rapidly and disappear within 24 hours. Numerous extracts of organisms have been introduced for this test such as *brucellin* (Olin 1935), *brucellergin* (Huddleson 1939), and purified brucella protein (Morales-Otero and González

growth. The characteristic of some strains to grow as small colonies, appearing as flakes attached to the side of the tube, is of some differential value. In any case, visible evidence of growth is very slight and almost all workers with these organisms have found it necessary to carry along an uninoculated tube of medium for comparative purposes.

The colonies upon an agar surface are usually not detectable until after two or three days' incubation. They are most readily observed in stained agar preparations. A square of agar is cut from a suspicious area on the plate and placed on a slide. It is covered with a coverslip on which an alcoholic solution of methylene blue and azure has been dried, and the space between the cover-

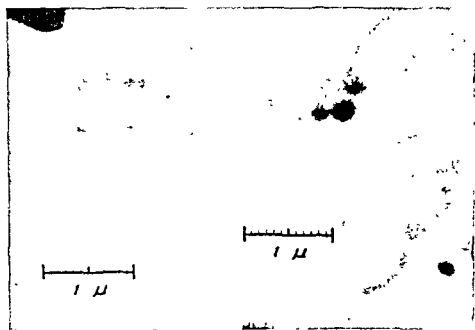


Fig. 120. Electron micrographs of the ring forms of the pleuropneumonia like organisms (Lilly Research Laboratories).

slip and the slide filled with melted paraffin.³⁹ The average colony size of different strains ranges from 0.01 to 0.6 mm., rarely exceeding the larger figure. The colonies usually have a dark center with a lighter margin, and may appear coarsely or finely granular. Upon higher magnification a foam like structure may be observed which is composed of the balloon like forms. Oily droplets may be found in the colonies of some strains, these have been found, in some cases at least, to be largely cholesterol already present in the medium.⁴⁰ Possibly the pseudo colonies which have been observed⁴¹ on uninoculated serum agar are of similar nature, it is said that these are readily differentiated from true colonies by experienced observers.

Physiology. These microorganisms require infusion or digest media enriched by the addition of serum or ascitic fluid in relatively large amounts

³⁹ Dienes, Ropes, Smith, Madoff and Bauer: *New England Jour. Med.*, 1948, 235: 509.

⁴⁰ Partridge and Kheneberger: *Jour. Path. Bact.*, 1941, 52: 219.

⁴¹ Brown, Swift and Watson: *Jour. Bact.*, 1940, 40: 857.

the specific complement-antibody system, and he therefore recommended the treatment of undulant fever with sulphadiazine or sulphamerazine by the mouth associated with the transfusion of human plasma or whole blood. Neither of these methods, however, is successful in more than a small proportion of cases (Molinelli *et al.* 1949). Highly favourable reports have been issued on the therapeutic effects of chloramphenicol and aureomycin. As many of these are based on the treatment of so-called chronic brucellosis, and as this disease is often diagnosed on inadequate grounds, it is difficult to assess the validity of the conclusions reached. In acute cases relapses seem to be frequent, and toxic manifestations may be disturbing (Spink 1950b, Harris 1950). According to Herrell and Barber (1950) good results are obtained with a combination of aureomycin and dihydrostreptomycin. An older method of treatment consists in the intramuscular or intravenous injection of brucella vaccines (see Schilling *et al.* 1931, di Guglielmo 1933) or the intramuscular injection of brucellin (Huddleson and Johnson 1933, Huddleson 1939, Debono 1935). The patient's sensitivity to *Brucella* is first gauged by a skin test, and the injections of vaccine or brucellin are given at intervals of 3 to 4 days in doses sufficient to evoke a smart febrile reaction. How far the effect is specific and how far it depends on protein shock therapy is doubtful. Serum treatment has been advocated (Hilgermann 1935), but is of questionable value (Mitchell *et al.* 1935, Gwatkin 1938).

BRUCELLA INFECTION OF SHEEP AND GOATS

Reference has already been made, under the section dealing with undulant fever due to *Br. melitensis*, to infections of sheep and goats with this organism, and to the diagnosis of the disease by examination of the blood serum and milk serum for agglutinins. The disease exists particularly in countries along the Mediterranean littoral, in South Africa, in parts, notably Arizona, of the United States, and in some areas of Russia (Karsten 1943). When first introduced into a herd, particularly during pregnancy, infection is frequently followed by a considerable number of abortions, but unless fresh animals are added, it dies down, and subsequent abortions are uncommon. The infection, however, usually becomes chronic, and may persist for months or years. In this stage it is often difficult to diagnose. The agglutination reaction may be negative. Some help is afforded by the intradermal meltin test. A suitable preparation, preferably a bacterial extract such as that described by Huddleson and Johnson (1933) or Taylor, Lisbonne, and Vidal (1935) is inoculated into one of the skin folds between the base of the tail and the margin of the anus. Readings are made after 24 and 48 hours. A positive reaction is characterized by œdema. Generally speaking, it may be said that a positive skin test indicates that the animal has been or is infected, and a positive agglutination test indicates that the infection is active. At post-mortem examination the organisms can be isolated most easily from the udder and the supramammary lymphatic glands. During life the organisms are often excreted in enormous numbers in the milk and the urine; after abortion the vaginal discharge may remain highly infective for some days. Infection probably occurs mainly by contamination of the skin and mucosæ, though possibly the alimentary route may play some part. It seems to be more persistent in goats than in sheep. In both animals the young are less susceptible than the sexually

Various workers add from 10 to as high as 40 per cent serum; 50 per cent and over is inhibitory for some strains. Some have included boiled blood and fresh serum, and small amounts of glucose facilitate primary isolation of some strains. In some cases these organisms tolerate a relatively wide pH range, but others die out at pH 7.0 and below and require a pH of 7.8 to 8.0 for growth. The saprophytic varieties will grow at 22° C. with an optimum at 30° C. but the parasitic ones require 37° C. Growth occurs both aerobically and anaerobically, but is less abundant with most strains under anaerobic conditions. Fairly heavy inocula are required, 0.1 to 0.2 ml. of minced tissue in primary isolation, the transfer of similar amounts of broth cultures, and in transfer from agar cultures a small section of the medium is cut out and dropped into liquid media or streaked on another plate. These organisms may also be cultured on the chorioallantoic membrane of the developing hen's egg.⁴² Some sugars, notably



Fig. 121. Colonies of a Type B pleuropneumonia-like organism from mice. Three days' incubation: $\times 100$. (Sabin, Bact. Rev.)

glucose, are fermented but the pH seldom drops below 7.0 owing to the death of the organisms at this point, the addition of other sugars may inhibit growth. In general, fermentations and other biochemical characteristics have no differential value.⁴³ The heat resistance of some strains is of the same order as that of most bacteria, while others appear to be more frail and are killed by exposure to 45° C. for fifteen minutes. Cultures are best preserved at incubator temperature in media containing no added sugar, when sealed with petrolatum the organisms may remain viable for a month or more.

Varieties, Strains or Species. Strains of these organisms have been isolated from a variety of sources. Those which are parasitic are definitely set off from the saprophytic types, and differentiation or identification within these groups has been made in part on source and pathogenicity, and in part on an immunological (agglutination) basis.

Bovine Pleuropneumonia. As indicated above, the causative organism of pleuropneumonia of cattle was the first of these forms to be isolated and

⁴² Swift. Jour. Exp. Med., 1941, 74:557.

⁴³ Cf. Warren Jour. Bact., 1942, 43:211.

the uterine mucosa and foetal membranes, resulting as a rule in the premature expulsion of the foetus. Though regarded as contagious by Hutrel d'Arboval (1826) and Youatt (1834) (see Hutyra and Marek 1922), its infective nature was not shown definitely till 1878, when Lehnert transmitted the disease by intravaginal inoculation of pregnant cows with the vaginal discharge and placental tissue of aborting animals. In 1897 Bang, working with Stribolt, demonstrated microscopically a small Gram-negative bacillus in the uterine exudate of a cow with impending abortion, and succeeded in isolating it in pure culture. The intravaginal injection of this organism into two pregnant cows gave rise to abortion, and from the uterine exudate the organism was recovered in each case. Bang's work was confirmed by Preisz (1903) and Nowak (1908) on the Continent, by M'Fadyean and Stockman (1909) in this country, and by MacNeal and Kerr (1910) in America.

Epidemiology and Bacteriology

The disease is widespread throughout Europe, the United States of America, and most other countries of the world where there is a large cattle population. Its exact frequency is difficult to ascertain, but in this country Priestley (1931) estimates that 20 per cent. of the cows are infected. Similar high estimates have also been made for certain areas in the United States (Cotton 1931, Thomson 1932, Birch 1934), but for the United States as a whole it is estimated that 5 per cent. of adult female cattle are infected distributed among 20 per cent. of the herds (Spink *et al* 1949b). Large herds are more often infected than small. On account of the heavy loss from abortions, the frequent subsequent sterility of the animals, and the diminution in the milk yield, it is economically one of the most important diseases affecting cattle (Minett and Martin 1936, Spink *et al* 1949b). The fact, moreover, that infection may be transmitted to man renders the disease of public health interest.

Though clearly it is only pregnant animals that can display the typical symptom of abortion, infection may be conveyed by natural channels to cattle of any age and either sex. There is no distinct seasonal incidence. The commonest time for abortion to occur is from the 5th to the 8th month of pregnancy; 35.4 per cent. of cases occur in the 7th month alone (Wall 1911). Judged from experimental work, the incubation period is variable; according to Mohler and Traum (1911) it may last from 1 to 33 weeks.

Introduced into a fresh herd the disease may spread rapidly, assuming epidemic proportions. Provided that no new animals are imported, it loses its initial severity, passing into an endemic state in which, if no preventive measures are taken, it remains for some years. A pregnant animal becoming infected for the first time generally aborts at an early stage; at its next pregnancy abortion occurs either not at all, or not till a later period; and though abortion may be repeated on a third occasion it is much commoner for the calf to be delivered at full term. Bang (1897) reports that of 83 cows only 30 aborted in 2 successive years, and only 6 three times in succession. From this it may be gathered that an immunity is acquired which is usually sufficient to protect the animal against further attacks of the disease.

The bacillus may, however, remain alive in the tissues of the cow for a considerable length of time. Bang (1897) found it in the uterus of a cow 9 months after the foetus had died and become mummified. As a rule the uterus frees itself

studied. The disease is found all over the world except in India, Western Europe and North America; it has been imported into this country a number of times but was finally eradicated by slaughter of infected animals and has not occurred since 1892.

The natural disease in cattle is characterized by extensive consolidation and subpleural effusion in either or both lungs and the microorganism is present in large numbers in the serous exudate. It spreads slowly in herds and may take an acute form with death within a week or a chronic form with walling off of the foci of infection. There is occasional joint involvement in young animals.



Fig. 122. The morphology of pleuropneumonia-like organisms. Both small and large enlargements, are shown as illustrated in the lower

The natural disease has not been reproduced in cattle by either inoculation of infectious serous exudate or of cultures, but an extensive edema develops and spreads from the site of inoculation, and there is a febrile reaction and sometimes death.

The organism is apparently completely non-pathogenic for the usual experimental animals and for man, it has been reported that cultures in sheep- or horse serum media are infectious for sheep and goats, while those in bovine serum media are not.

The name *Asterococcus mycoides* was given the organism by Borrel and Dujardin Beaumetz but has not been generally used. Sabin³⁶ has proposed the name *Bovimycetes pleuropneumoniae*. In any case, strains obtained in different localities and at different times appear to be very similar if not identical.

seems to be affected more often than the left (Bang and Bendixen 1931, Thompson 1934, Doyle 1935).

Though the organism commonly responsible for the disease is *Br. abortus*, numerous observations have shown that cattle in close contact with infected goats or sheep may become infected with *Br. melitensis* (see Taylor, Vidal, and Roman 1934, Benussi 1935). The infection seems to be localized mainly in the udder, and abortion is uncommon. Similarly in large hog-raising districts, cattle may become infected with *Br. suis* and excrete this organism in the milk (see Beattie and Rice, 1934, Mohnelli and Ithurrat 1934), but, as with *Br. melitensis*, the disease produced tends to be more self-limiting than that caused by *Br. abortus*.

Mode of Infection and Experimental Reproduction of the Disease.—There has been a long dispute over the commonest mode of infection. Cattle are so easy to infect experimentally, not only by direct inoculation into the tissues, but also by the natural passages, that it is very difficult to decide on the route usually followed in the natural spread of disease. The three main portals are:

(1) **THE MOUTH**.—Bang (1906) and others showed that it is possible to reproduce the disease in pregnant heifers and cows by feeding them with infective material, and therefore suggested that infection probably occurs through contaminated fodder. It is known that the uterine exudate which is voided at the time of the abortion is extremely infective, that the bacilli may remain alive in it for some time—for 7 months if kept in the ice-chest (Bang 1897); that the aborted foetus or foetal membranes deposited on grass and left at atmospheric temperature shielded from direct sunlight may remain infected for as long as 6 months (Bosworth 1934–35); and that unless strict isolation of the animals is practised, there is ample opportunity for contamination of the pasture and other food material. A further source of infection is the milk of the aborting cows, which often contains *Br. abortus* in large numbers. Rats have been accused of spreading infection (Karkadinovsky 1936), but there is little evidence to support this (see Bosworth 1940).

(2) **THE VAGINA**.—In his original experiments Bang (1897) demonstrated the possibility of infecting pregnant cows by intravaginal inoculation of pure cultures. This has been confirmed frequently (Report 1909, M'Fadyean *et al.* 1913, Seddon 1919). It seems, therefore, not improbable that contamination of the vagina with infected soil or litter may be responsible for naturally acquired infection. A second method by which vaginal infection may occur is sexual intercourse. Bulls may be infected by injection of pure cultures under the prepuce (M'Fadyean *et al.* 1913). Injected intravenously, the organisms may lodge in the testicles and be excreted in the seminal fluid (Seddon 1919). Infection of bulls is not uncommon under natural conditions, and lesions may occur in the testis, epididymis, or vesiculæ seminales. Bendixen and Blom (1947), who draw attention to the danger of transferring infection to the cow by artificial insemination, isolated *Br. abortus* from the semen of 28 out of 394 bulls. There is also epidemiological evidence to incriminate the bull as a means of spreading the disease (Bang 1897).

(3) **THE SKIN AND CONJUNCTIVA**.—Attention has been drawn to these routes of infection by Bang (1931), Bang and Bendixen (1932b), Cotton and Buck (1932), and Cotton, Buck and Smith (1933). Experimentally it has been found possible to infect animals fairly readily with *Br. abortus* by means of an infected compress left for a variable length of time in contact with the abraded, or even intact, skin. Infection may also follow simple conjunctival instillation of an *abortus* suspension.

Which of these three routes is the most important in practice, we have at present no exact means of telling. The Departmental Committee set up by the Board of Agriculture to investigate the disease considered that feeding was the most common method, that vaginal infection held second place, and that sexual infection probably played quite an insignificant part. Contamination of mucosal and skin

Contagious Agalactia of Sheep and Goats. Despite its name this disease is a generalized infection which affects both males and females. It occurs only in parts of southern Europe and North Africa; agalactia or mastitis of these animals in this country is of other etiology.

The lesions occur in the joints and eyes and in the mammary glands of females. The microorganism is present in the blood early in the disease and later may be isolated from the affected regions and from the mammary secretions. The second member of the group to be studied, it was isolated by Bridré and Donatien in 1923. It is morphologically and biochemically very similar to the pleuropneumonia organism and other members of the group, but is immunologically distinct and of characteristic pathogenicity. Sabin³⁶ has suggested that it be called *Capromyces agalactiae*. The disease is readily reproduced by inoculation of sheep and goats with cultures.

Canine Variety. Organisms of the pleuropneumonia group have also been found in dogs. Shoetensack reported in 1934 the cultivation of an organism of this type from the purulent nasal discharge of dogs ill with distemper which he called *Asterococcus canis*. In later studies other organisms were found and there appeared to be two types, immunologically distinct from one another, which he called Type I and Type II respectively. Sabin³⁶ proposed that these be called *Canomyces pulmonis I* and *Canomyces pulmonis II*. Their postulated etiological relationship to distemper, generally regarded as a virus disease, is not established.

Varieties from Rats. A series of pleuropneumonia-like organisms has been isolated by Klieneberger and her associates⁴⁴ from the respiratory tract and elsewhere in normal and diseased rats. Others have isolated similar organisms from rats exhibiting polyarthritis and swollen extremities. These comprise, with a single exception, the "L" series of strains in which the strains are designated by subscript numerals. There now appear to be three distinct types, differentiated on a biological and immunological basis, viz., L₁, L₃ and L₄. The strain L₂ was isolated from a guinea pig and insufficiently studied before loss of the culture at the outbreak of war. The strain described as L₅ is immunologically identical with mouse type A discussed below; L₆ has been insufficiently studied; and L₇ has been found to be identical with L₄.

Considerable interest has attached to the apparent association of L₁ with *Actinomyces muris-ratti*,⁴⁵ Klieneberger has been able to isolate L₁ from many strains and stock cultures of *A. muris-ratti* and it will be recalled that this fungus is a natural parasite of the nasopharynx of rats and mice. It is maintained by Klieneberger⁴⁶ that L₁ and the fungus coexist as symbionts since she has been able to separate the two on the basis of differential resistance to heat and aging, has observed L₁ in the rat in the absence of *A. muris-ratti*, and has carried L₁ strains through many transplants without reappearance of the

⁴⁴ Klieneberger: Jour. Path. Bact., 1935, 40 93; *ibid*, 1936, 42 587; Klieneberger and Steabben. Jour. Hyg., 1937, 37-143, Klieneberger. Jour. Hyg., 1940, 40 204.

⁴⁵ It may be noted that the name *Streptobacillus moniliformis* continues to be used exclusively in the pleuropneumonia group literature in spite of its impropriety and almost always with no mention of its synonyms. The unnecessary confusion introduced is unfortunate. See page 670.

⁴⁶ In several papers, Cf. Klieneberger: Jour. Hyg., 1942, 42 485.

the material is not pure, it is best to resort immediately to guinea-pig inoculation. The material is ground up in Ringer's solution, and 0.5 ml. injected intramuscularly into two or three guinea-pigs. The animals are killed after 4-8 weeks, the blood is examined for agglutinins, and cultures are made from the sublumbar glands and the spleen (see "Milk" below). At post-mortem examination of slaughtered cattle the organisms are most likely to be found in the udder, and in the supramammary, iliac, and pharyngeal glands. They may be sought for by direct culture and by guinea-pig inoculation (see Doyle 1935). Any obvious lesions, such as hygroma of the knee (v. d. Hoeden 1932a), should also be examined.

Agglutination Reaction.—In practice this is the most widely used method of diagnosis. Although the agglutination test in cattle does not differ in principle from that in man, it is necessary to consider separately its interpretation in relation to the titre that may be observed.

The test may be carried out by the tube or the slide method (for technique, and preparation of antigens, see Huddleson and Abell 1928, Huddleson 1932, 1939, Welch and Mickle 1933, Fitch and Donham 1933, Donham and Fitch 1934a, b, 1935, Welch and Marsh 1935). Final serum dilutions of 1/10-1/2560 should be put up. Intermediate zone phenomena are frequently encountered (see Priestley 1931), and agglutinin-blocking bodies interfering with tube but not with slide agglutination have been described (Cox and Kutner 1950). The ordinary zone phenomenon can be avoided by the use of 5 per cent. saline.

The interpretation of this test is often difficult, and is not rendered any easier by the fact that, owing to differences in technique, the titres of different workers may not be strictly comparable. Comparable results, however, may be obtained by the use of a standard agglutinating serum, which can be used in the characterization of routine agglutinating suspensions and in the specification of routine methods of performing the test. An International Standard for Anti-*Brucella abortus* Serum is available for this purpose (Stableforth 1954).

Calves, even those born of infected mothers, give a negative serum reaction at birth. If, however, they are allowed to suck infected dams within the first 24 hours of life, agglutinins become demonstrable in their serum within about 2 hours. This appears to be due to the absorption through the alimentary tract of antibodies present in the colostrum. Calves passively immunized in this way lose their agglutinins very rapidly (McAlpine and Rettger 1925). Even calves that are actively infected and develop agglutinins some time after birth appear to lose them within 6 months, unless reinfection occurs (Thorp and Graham 1933).

In an animal that is infected during pregnancy the titre generally rises before abortion to 1/200-1/1000 or over. During the following 6 months it tends to fall. If the cow becomes a chronic carrier, the titre usually remains fairly high. About 80 per cent. of cows with a persistent titre of 1/200 or over are found to be excreting *Br. abortus* in the milk. A titre of 1/1000 or over is almost diagnostic of udder infection. If animals are followed for any considerable length of time, the agglutinin titre of positive reactors will often be found to decline. This is particularly true of low reactors. Damon (1932), for example, who observed a herd with a mean complement of 225-250 animals over a period of 4 years, found that 27.7 per cent. of animals reacting at 1/25, 17 per cent. of those reacting at 1/50, and 5 per cent. of those reacting at 1/100 or over lost their agglutinins permanently (see also Hadley and Welsh 1931). Huddleson and Smith (1931), however, found that, of a total of 247 animals reacting at 1/25 or over, and followed up for a period of 1-8 years, only 4 became permanently negative. Animals that abort usually have a considerably higher titre than non-aborting animals. A few

actinomycete. Dienes,⁴⁷ however, has taken the view that *L*₁ and *A. muris-ratti* represent but stages in a cyclogeny.

These *L* forms are associated, causally in some instances at least, with relatively mild, chronic affections of rats in which a not uncommon manifestation is joint involvement and polyarthritis. They closely resemble the other organisms of the group morphologically and culturally, forming a subgroup on the basis of pathogenicity, and differentiated from one another immunologically. Sabin³⁶ has grouped *L*₁, *L*₃, and *L*₄ under a single genus and suggested the names *Murimycetes streptobacilli-moniliformis*, *Murimycetes pulmonis* and *Murimycetes arthritidis* respectively for them.

Varieties from Mice Sabin³⁵ divides the pleuropneumonia-like organisms found in mice into five types, designated as A, B, C, D and E. Type A is found in normal mice, once in the brain and frequently in the eyes, nasal mucosa and lungs of carriers. On intracerebral inoculation of mice an ataxia is produced which is characterized by a turning or rolling of the body. The brain lesions and symptoms arising from them are attributable to the action of a soluble toxin produced by the microorganisms.⁴⁸ An immunologically identical organism was isolated by Findlay *et al.* from mice affected with "rolling disease" and designated *L*₅. Type B has likewise been found in normal mice and is not only immunologically distinct but produces a progressive arthritis almost exclusively when parenterally inoculated into mice. Types C, D and E are similar in their pathogenicity but are immunologically distinct, they have not been studied as extensively as some of the others. These murine forms are, like the *L* forms, grouped in a single genus by Sabin,³⁶ type A being given the name *Musculomycetes neurolyticus*, type B the name *Musculomycetes arthrotropicus*, and types C, D and E grouped in the single species *Musculomycetes histotropicus*.

*Pleuropneumonia like Organisms of Man.*⁴⁹ Though there is considerable evidence suggesting that certain streptococci are associated with some types of arthritis in man (p. 377), the etiology of human arthritis is far from clear. The pleuropneumonia-like organisms have assumed some interest in this connection, largely because of the frequency of occurrence of progressive polyarthritis in both naturally and artificially infected rats and mice. The association of *L*₁ strains with *A. muris-ratti* is of possible interest in view of the arthritic manifestations of epidemic disease of the Haverhill fever type and of sporadic rat-bite fever of fungous etiology (p. 670).

Microorganisms of this group have been found by a number of workers in the genital tract. They appear to occur with some frequency in the normal vagina and cervix, in 58 of 222 routine specimens in the series reported by Dienes *et al.*,⁴⁹ but they have also been found associated with inflammatory conditions of the cervix. In the male, however, they have been found largely in association with pathology of the genito-urinary tract. Dienes *et al.* reported that all of 58 patients from whom cultures were recovered had urethritis.

⁴⁷ Dienes. Jour. Inf. Dis., 1939, 65: 24. Also Heilman: *ibid.*, 1941, 69: 32, 45.

⁴⁸ Sabin. Science, 1938, 85: 189, 575.

⁴⁹ See the review and discussion by Dienes, Ropes, Smith, Madoff and Bauer. New England Jour. Med., 1948, 238: 509, 567.

intermittent and that not too much attention should be paid to a single negative result.

Cultivation.—Cultural methods are satisfactory only with milk samples from individual cows collected under more or less aseptic conditions. Separate quarter samples should be taken, and either kept refrigerated, or preserved with 0.5–1.0 per cent. boric acid, till the time of examination. The whole milk, the gravity cream, or the deposit from high-speed centrifugation should be streaked on to a number of plates of liver extract agar, or 2 per cent. glycerol agar containing 10–15 per cent. of ox serum. Contaminating organisms may be partly suppressed by the addition to the medium of gentian violet and malachite green. The exact concentrations of these dyes vary according to the manufacturer; usually a final concentration of 1/100,000 to 1/200,000 is satisfactory. The plates should be incubated in 5–10 per cent. CO₂, and examined at intervals for 14 days (see Huddleson *et al.* 1927, Hasley 1930, Traum and Henry 1930, Karsten 1932, Proscholdt 1932, Karsten and Bischoff 1933, 1934, Stockmayer 1933a, 1935). A method introduced more recently by Mair (1954) makes use of blood agar containing not only gentian violet to keep down unwanted organisms, but penicillin and polymyxin in addition, together with acti-dione for the suppression of moulds. On this medium *Br. abortus* may be isolated even from herd milks.

Animal Inoculation.—If the animal method is chosen, it is advisable to inoculate a mixture, composed of 2 ml. of gravity cream and the deposit from 100 ml. of milk after high-speed centrifugation, intramuscularly into the hind leg of each of two guinea-pigs. Alternatively 4 ml. of whole milk may be injected, 2 ml. into each thigh. The animals should be killed about 6 weeks later. At post-mortem examination the femoral and sublumbar glands will be enlarged and pale; the spleen may be enlarged, its surface slightly irregular, and a few small greyish-yellow necrotic foci may be present. The liver may show two or three tiny necrotic foci. The macroscopic lesions are often inconspicuous, and must on no account be relied upon for diagnosis. The blood serum should be tested for agglutinins; a titre of 1/25 or over is highly suggestive of infection. Cultures should be made from the sublumbar glands and spleen, and all suspicious organisms identified by agglutination and other methods. In not all animals containing serum agglutinins is it possible to isolate the organisms from the tissues. On the other hand, it is uncommon to isolate them in the absence of a positive agglutination reaction. Individual guinea-pigs vary considerably in their susceptibility to *Br. abortus*, and it is common to obtain positive results in one animal and negative in the other (see Smith 1932b, Plate 1934a). If tubercle bacilli are present in the milk simultaneously with *Br. abortus*, the guinea-pig will suffer from a double infection. The diagnosis of tuberculosis can be made on the basis of the macroscopic lesions and the demonstration of acid-fast bacilli in the organs. The diagnosis of *abortus* infection can be made on the basis of the agglutination reaction and the cultivation of the organisms from the tissues. Pullinger (1936), however, pointed out that the tubercle bacillus interferes with the development of *Br. abortus* in the animal body, so that the demonstration of this organism may often be unsuccessful in milk coming from cows with tuberculous mastitis.

All workers are agreed that the animal inoculation method is more satisfactory for the demonstration of *Br. abortus* in milk than the direct cultural method. Plate (1934b) found that 90 per cent. of infected samples were positive by the animal and only 50 per cent. by the cultural method. Similarly Karsten and Bischoff (1934), who examined 466 milks by both methods, obtained 184 positive results by the guinea-pig and 101 by the cultural method. It is not uncommon to obtain occasional positive results by culture, when the animal inoculation results are negative, so that it is advisable to use both methods. In mixed milk, or in milk of single animals that has not been drawn aseptically and kept cold, the animal inoculation method is the only suitable means of demonstrating *Br. abortus*.

prostatitis or cystitis. In relation to the possible association of these microorganisms with arthritis in man, it is of interest that 18 of this series had an acute type of arthritis, 9 had simultaneous urethritis, conjunctivitis and arthritis, the syndrome characteristic of Reiter's disease, and in two instances pleuropneumonia-like organisms were recovered from synovial fluid. Observations such as these suggest that they may be pathogenic for man.

Saprophytic Varieties. Pleuropneumonia-like organisms presumably living a saprophytic existence in nature have been found by Laidlaw and Elford⁵⁰ in raw London sewage. They closely resembled the parasitic forms both morphologically and culturally, and fell into three immunological groups which were designated types A, B and C. All were non-pathogenic for experimental animals. Similar forms have been found in Germany⁵¹ in compost and other types of decomposing organic matter. Sabin⁵² has proposed that these saprophytic forms be named *Sapromyces laidlawi*.

DONOVANIA GRANULOMATIS

The disease granuloma inguinale (granuloma venereum) is not to be confused with lymphogranuloma inguinale of virus etiology (p. 882). It is characterized by a slowly progressive ulceration in the genital region and rarely elsewhere. The initial lesion is a swelling, often in the groin as a bubo, which ruptures. Daughter lesions appear which are at first discrete and then spread slowly and coalesce, and the process may eventually involve the skin of the groin, genitals, buttocks and lower abdomen and the patient develops a strong fetid odor. Little effective immunity appears to be developed, at least not sufficient to appreciably arrest the progress of the infection.

Bacillary bodies, stained by Wright's stain, were observed by Donovan in 1905 in smears from lesions or in biopsy material, and have long been known as Donovan bodies. The Donovan body has been cultivated in the yolk sac, but not on the chorioallantois, of the developing chick embryo by Anderson, De Monbreun and Goodpasture⁵² and in enriched media such as beef heart infusion by Dunham and Rake,⁵³ and is thus shown to be a cultivable microorganism to which the name *Donovania granulomatis* has been given.

The morphology of this microorganism in culture has been studied by Rake and Oskay,⁵⁴ and it is described by these workers as a short, plump bacillus 1.5 μ to 4.5 μ in length and 0.8 μ to 1.4 μ in breadth, gram-negative and showing prominent polar granules. Prior to cell division the elongated bacillary forms tend to become curved, and may remain attached after division to give rise to chains of bacilli, the coiled filaments often seen in the usual stained preparations. The relatively heavy encapsulation observed in preparations from lesion material and the mucoid character of yolk sac cultures and initial cultures on artificial media diminish with continued culture. Aside from its highly fastidious growth requirements, *D. granulomatis* closely resembles Friedlander's

⁵⁰ Laidlaw and Elford: Proc. Roy. Soc. (London) Ser. B, 1936, 120:292.

⁵¹ Seiffert: Centralbl. f. Bakt., I Abt. Orig., 1937, 139:337; *ibid*, 1937, 140:168.

⁵² Anderson, De Monbreun and Goodpasture: Jour. Exp. Med., 1945, 81:25.

⁵³ Dunham and Rake: Amer. Jour. Syph. Gonorrhea Ven Dis., 1948, 32:145.

⁵⁴ Rake and Oskay: Jour. Bact., 1948, 55:667.

principle consists in the detection of infected animals by the agglutination, complement-fixation, or Ring test, the slaughter, sale, or segregation of positive reactors, and the building-up of a clean, non-infected herd. The degree of success attending this policy varies with a number of factors. With a small or medium-sized herd, particularly if self-contained and protected against infection from water, manure, and other animals, with provision of calving boxes, and re-testing of non-reactors every 2 months so as to eliminate all animals as soon as they become positive, it is often possible to eradicate the infection entirely and to maintain a healthy herd for several years. On the other hand, with very large herds, particularly if not self-contained, with flying herds, with farms on which adequate accommodation for segregation of infected, and quarantine of newly imported, animals is impossible, and with imperfect control over infection from other sources, the results are often disappointing. Generally speaking, if the conditions are favourable, and if the disease is not at the height of its activity, this policy should be adopted, since its success is followed by the improved general health of the herd, better and more regular breeding, and an increased milk supply. Success, however, cannot be guaranteed. One of the greatest problems at the moment is presented by the infected non-reactor, which may abort, or excrete *Br. abortus* in the milk, and so contaminate the other animals in the herd (see Bang 1906, M'Fadyean 1921, 1924, Giltner 1924, Thomsen 1928, Zeller 1931, Henricsson 1932, Kitzelman 1932, Newsom and Cross 1932, Clark 1932, Mitchell *et al.* 1933, Fritz and Barnes 1933, Birch 1934, Birch *et al.* 1934, van Oyen 1934, Report 1935, Thornshaug 1937, Gilbert 1937, Spink *et al.* 1949b, Gilman 1950).

Vaccination.—Early observations by Bang (1906), M'Fadyean and Stockman (1909) and Stockman (1914) suggested that the inoculation of non-pregnant heifers or cows with a living vaccine of *Br. abortus* was able to confer some degree of immunity on the animals, as shown by a lower abortion rate. During the next 20 years or so vaccination, usually with strains of modified virulence, was used fairly extensively, and numerous papers were published on the results (for references, see Topley and Wilson, 2nd ed., p. 1355). Little fresh knowledge, however, was gained, chiefly because few observations with properly controlled groups of animals were made. More recently a great deal of attention has been devoted to the preparation of a standardized vaccine, and much valuable information has been forthcoming. It has been found that the more virulent the vaccinal strain is, the greater is the degree of subsequent immunity; but that in practice the use of a fully virulent strain is inadvisable, because of the danger partly to pregnant animals in the herd and partly to the human population if it is excreted in the milk. A strain of modified, and preferably fixed, virulence is desirable. Such a strain was introduced by Buck (1930) in the United States under the name S19, and has since been used with good effect (for references, see Haring 1933, 1939, Haring and Traum 1937, 1941, McEwen 1941, Huddleson 1942, Crawford 1947, Traum 1950). The vaccine is used for calves preferably 7–12 months after birth, for non-pregnant heifers, and for cows not more than 4 months pregnant. Revaccination of animals vaccinated in calfhood is desirable after the first parturition.

One disadvantage of the S19 strain is that it stimulates the formation of antibodies and thus interferes with the normal interpretation of the agglutination test; though if the animal is vaccinated during calfhood the agglutinins usually disappear within 6–18 months.

bacillus, and has been found by Rake⁵⁵ to be closely related immunologically to this bacterium and to *Bact. coli* and *Bact. aerogenes*.

Prior to the isolation in pure culture of *D. granulomatis*, its causal relation to granuloma inguinale was only suggested by association. It was shown by Anderson, Goodpasture and De Monbreun⁵⁶ that material from yolk sac cultures gave a skin reaction in persons having the disease, and a mucoid substance from infected yolk sac, possibly a polysaccharide, gave precipitin and complement-fixation reactions with sera from patients. The specificity of these reactions is, however, open to question in that apparently specific complement fixation was given by persons with chronic, non-specific ulceration (viz., varicose) studied by Rake and his co-workers and attributed by them to the serological relation of *D. granulomatis* to coliform bacilli. Later Greenblatt *et al.*⁵⁷ were able to produce the disease in two human volunteers, one inoculated by a subcutaneous transplant of biopsy material, and the other by the subcutaneous inoculation of yolk sac culture. Though it has not yet been possible to infect experimental animals with *D. granulomatis*, it seems established that this microorganism is the etiologic agent of granuloma inguinale.

The general tendency to regard this disease as venereal is based in large part on the location of the lesions. There is little or no direct evidence that it is transmitted primarily by sexual contact and, in fact, its occurrence in both marital partners is uncommon. The probable incubation period of one to four weeks is not excessively long and should not greatly obscure histories of contact. It has been suggested that there is great individual variation in susceptibility and that natural resistance is usually of a high order. The disease is associated with uncleanness and in this country occurs for the most part in the Negro of low economic status in the southeastern States, but it occurs elsewhere also. It is estimated that there are 5000 to 10,000 cases in the United States and it has been found to constitute 2 to 3 per cent of venereal disease in Negro recruits. In general, however, the epidemiology of granuloma inguinale is as yet very poorly understood.⁵⁸

⁵⁵ Rake Jour. Bact., 1948, 55 865.

⁵⁶ Anderson, Goodpasture and De Monbreun Jour. Exp. Med., 1945, 81 41.

⁵⁷ Greenblatt, Dienst, Kupperman and Reinsteim Jour. Ven. Dis. Inf., 1947, 28 183.

⁵⁸ See the discussion by Clarke Jour. Ven. Dis. Inf., 1947, 28 189.

than 269 out of 786 boars showed orchitis, usually bilateral. The affected testicles are sometimes of enormous size, reaching 6½ lb. in weight, and purulent foci, which may undergo calcification, are not infrequent. Multiple foci may also be present in the vesiculæ seminales. *Br. suis* may, however, often be demonstrated in the tissues in the absence of macroscopic pathological changes.

In sows a condition for which Thomsen (1934) suggests the name "miliary brucellosis of the uterus" is frequently met with. The interior of the uterus is studded with small yellowish-white nodules located in the deeper layer of the mucosa and projecting slightly above the surface.

The disease can be readily reproduced by feeding and by conjunctival inoculation. According to Stockmayer (1933b), pigs are susceptible to *Br. abortus* as well as to *Br. suis*, though no outbreaks due to *Br. abortus* have yet been recorded in the field. Thomsen believes that natural infection is spread to a considerable extent by copulation, though this of course will account only for transmission of the disease to sexually mature animals. Infection of the conjunctiva by urine, and alimentary infection probably play a part. Sucking pigs are readily infected. It is possible that infection occurs through the milk of the mother, though invasion of the udder is uncommon.

In the field, *diagnosis* is best made by the agglutination and complement-fixation tests on the blood serum. A titre of 1/100 is regarded as definitely positive, 1/50 as strongly suggestive, and 1/25 as doubtful. Some help may be given by the intradermal test (Thomsen 1934). In the abattoir, the blood serum should be examined, and a search made by culture and guinea-pig inoculation for *Br. suis* in the tissues. According to Johnson and Huddleson (1931) and Johnson, Huddleson, and Hamann (1933), the organisms are commonest in the spleen, gastric and supramammary lymph glands, and the liver. Numerous other organs may be infected, but less frequently.

The disease is of undoubted economic importance, owing particularly to impotence in the boars and abortion or sterility in the sows. But it is probably of even more importance in its effect on public health. Already large numbers of cases of undulant fever have been ascribed to it, and it affords a very real hazard for workers in abattoirs and packing houses. So far as reports go, it would seem to be fairly amenable to control by the eradication policy. In Denmark, by the institution of such a policy, including slaughter of infected animals in lightly infected herds, and slaughter of all animals in heavily infected herds, it proved possible to stamp out the disease entirely within a very short time. Johnson, Huddleson, and Hamann (1933) in the United States were successful with a less radical policy, consisting of blood testing once a month, followed by segregation and ultimate elimination of the positive reactors. The seasonal breeding of sows makes control of the disease considerably easier than in cattle. Attempts at prophylactic vaccination have hitherto been a failure. (For further information on this disease the reader is referred to the excellent monograph of Thomsen 1934.)

BRUCELLA INFECTION OF HORSES, DOGS, CATS, FOWLS AND RATS

Horses.—In sequence to the work of Riniard and Hilger (1928) in France, *Brucella* infection of horses was rec
van der Hoeden 1931, Makkawejsky : . . .

BACILLUS—THE SPORE-FORMING AEROBES

The spore-forming rod-shaped bacteria are divided into two groups on the basis of their relation to atmospheric oxygen. The Bacilli are the aerobic forms and the anaerobic types are designated Clostridium (Chap. 28). A very large number of species of Bacilli have been described, the majority of them from soil and dust. Two species, *Bacillus alvei* and *Bacillus paraalvei*, cause foulbrood, a disease of bees. *Bacillus subtilis* infects human beings only rarely, and, with this exception, *Bacillus anthracis* is the only member of this large group that is pathogenic for man.

BACILLUS ANTHRACIS

Primarily a disease of lower animals transmissible to man, anthrax (splenic fever, Fr. *charbon*; Ger. *Milzbrand*) is of particular historical interest, for it was in his study of this disease that Koch provided the first demonstration of the causal relation between a specific bacterium and an infectious disease. The bacillus had been observed in the blood and organs of animals dying of anthrax by Davaine and Rayer in 1850 and by Pollender in 1855. In 1887 Brauell transmitted the disease by the inoculation of blood from infected animals. Conclusive demonstration of the causal relation between the bacilli and the disease, however, was the work of Koch, who in 1877 cultured the bacillus on the aqueous humor of the ox's eye, described its life history, and reproduced the disease with a pure culture of the microorganism. The importance of this discovery to the development of bacteriology has been discussed elsewhere (Chap. 1).

Morphology and Staining. The anthrax bacillus is one of the largest of the pathogenic bacteria and ranges from 4.5 to 10 μ in length and from 1 to 1.25 μ in breadth. The ends of the rods are often concave and somewhat swollen so that the appearance of a chain of anthrax bacilli has often been compared to a jointed bamboo fishing rod. The cells occur singly and as end-to-end pairs or short chains in the body, but in culture long chains are formed. Unlike most of the sporulating aerobic bacilli they are non motile.

Capsules may be found on the bacilli in smears from an infected animal but are not found in culture except on media rich in animal protein, such as serum agar. The capsular material is not polysaccharide as it is in most bacteria, but is a high molecular weight polypeptide composed exclusively of d(-)glutamic acid (the "abnormal" stereoisomer).¹ This is a point of particular interest, for it is the first demonstrated natural occurrence of d(-)glutamic acid and of a polypeptide composed of a single amino acid. There is, in addition, a poly-

¹ Cf. Hanby and Ryden. *Biochem. Jour.*, 1946, 42 297

Cats.—Little is known about the incidence of *Brucella* infection in cats, though considering their habit of drinking raw cows' milk it is difficult to believe that they are not frequently infected. Experimentally, both *Br. abortus* and *Br. melitensis* may give rise to a severe disease with lesions in the joints and internal organs (Makkawejsky and Karkadinowskaja 1932).

Fowls.—The frequency of *Brucella* infection in fowls is still under discussion. Emmel and Huddleson (1929, 1930) brought evidence to suggest that the disease was very common in the United States, but their observations have not been altogether confirmed by subsequent workers (McNutt and Purwin 1930, v. Roekel *et al.* 1932). It is noteworthy that their conclusions were based on the presence of agglutinins in the blood. The isolation of the organisms from the tissues of naturally infected birds seems to have been very rarely accomplished. Beller and Stockmayer (1933a) showed that normal fowls frequently have agglutinins in their blood, and that no attention can be paid to a titre of less than 1/150 or 1/200. Most workers have found that experimental inoculation of fowls gives rise to an inapparent infection, having little or no effect on the health or egg-laying capacity of the birds (see McNutt and Purwin 1930, 1932, v. Roekel *et al.* 1932, Lombardo 1932, Beller and Stockmayer 1933a, b, and Chapter 34). The natural infection seems to be of little epidemiological or clinical interest, though further observations are required, particularly to find out whether fowls may transmit infection to cattle.

Rats.—Not much is known of the occurrence of natural infection in wild rodents, though, in view of the possible part played by these animals in the spread of infection among cattle, knowledge is urgently required. Karkadinovsky (1936) examined 31 wild grey rats on three farms infected with contagious abortion, and isolated *Br. abortus* from 11 of them. Most of the positive cultures were from the spleen and liver, but in two of the animals the blood was also found to be infected. Bosworth (1940), on the other hand, who examined 167 rats caught on farms where the disease was prevalent, found only one harbouring *Br. abortus*. The observations of Bosworth (1937) and of Sandholm (1938) show that rats can be infected by feeding, and that the organisms can be excreted for a short time in the faeces and urine, but that on the whole the animals are relatively resistant and that tissue lesions seldom occur.

INFECTIOUS ABORTION IN CATTLE AND SHEEP DUE TO *Vibrio fetus*

In 1913 M'Fadyean and Stockman (Report 1913) described an enzootic abortion of sheep due to a spirillar organism, which was later called *Vibrio fetus* by Smith (1918). In this country, vibronic abortion appears mainly to affect sheep, though the disease has been observed in cattle; but in the United States it is responsible for a considerable proportion of cases in cattle. Smith (1919) found, for example, in 109 cases of abortion in which a fairly thorough examination of the fetus and membranes was made, that 62 were due to *Br. abortus*, 26 to *V. fetus*, 2 to *C. pyogenes*; in the remaining 19 no organisms were isolated or mixed cultures were obtained. In cows the agglutination test is of value in diagnosis, a serum reaction of 1/200 being regarded as positive (Plastring and Williams 1913). In

usually disappear from the serum within 6 months. The vibrios are com-

saccharide haptene present in the cell substance of the bacilli, which may be isolated from these bacilli, it appears to be the same in both virulent and avirulent strains and its immunological function is not clear.²

The anthrax bacillus also differs from most other aerobic pathogenic bacteria in that it forms spores which are visible as refractile bodies either free or located centrally within the cell. Their diameter does not exceed that of the vegetative cell and hence the spore-containing rod is not distorted. Spores are formed most abundantly at 32° to 35° C and only under aerobic conditions, i.e., not in the circulating blood of infected animals. Germination of the spore is usually polar, that is, parallel with the long axis, but may be rarely equatorial.

The bacilli stain readily, but often unevenly, with the usual aniline dyes. The granular material within the cell consists of fat, volutin or glycogen.



Fig. 123. *Bacillus anthracis*, forty eight hour culture on nutrient agar. Crystal violet stain. The spores appear as unstained areas. Note the typical arrangement of the bacilli in coiled chains. $\times 1200$

They are gram positive. Churchman³ has observed a reversal of the Gram reaction when aqueous gentian violet, acriflavin or acriviolet is added to suspensions of young cultures of *Bacillus anthracis*, a considerable proportion of the bacilli then become gram negative. It has been concluded that the bacillus is made up of a gram positive outer layer or cortex and a gram negative medulla. When the former is destroyed the latter comes into view and the gram negative bacilli are considerably smaller than the gram positive cells. The spores are stained with difficulty, and after staining with hot carbol fuchsin are equally difficult to decolorize, hence the vegetative cells may be decolorized and stained with a contrasting dye.

The colonies of the anthrax bacillus are irregular and have a curled or hair-like structure, giving what is sometimes called a "Medusa head" appearance. On microscopic examination tangled coils of long chains of bacilli may be found. This colonial appearance is closely simulated by *Bacillus subtilis* and some other related saprophytic, aerobic, spore forming bacilli.

Kanovsky. Ztschr. f. Immunitätsf., 1940, 97 443.

Churchman. Jour. Exp. Med., 1927, 46 1007.

in America, particularly in the Western States; ground-squirrels, hares and jack-rabbits are the animals chiefly infected. Burroughs and his colleagues (1945) give a list of 48 mammals and birds in which natural infection is known to occur. The lesions found in animals dead of the disease are similar to those of plague; there is a bubo, generally in the cervical, axillary, or inguinal region, containing dry, yellowish, caseous material; the spleen is greatly enlarged, very dark in colour, and contains yellowish-white, discrete, caseous granules up to 1 mm. in diameter, projecting slightly above the surface; there are numerous granules in the liver; the lungs are rarely affected; the organisms are present in enormous numbers in the spleen, in smaller numbers in the liver, bubo, and heart's blood (McCoy and Chapin 1912). Experimentally the disease can be reproduced in ground-squirrels, gophers, guinea-pigs, rabbits, mice, and monkeys; rats are more resistant, cats, dogs, and pigeons appear to be immune. Feeding, nasal instillation, cutaneous, subcutaneous, intraperitoneal, and conjunctival injection are all successful (see Chapter 31.) The disease appears to be spread by blood-sucking insects, especially ticks (McCoy and Chapin 1912, Francis 1921, Francis and Lake 1922, Parker *et al.* 1921, 1929). According to Parker and Spencer (1926*b*) the organism may be transmitted from infected female ticks to their progeny; if this is true, it is one of the few examples known of the hereditary transmission of a bacterium by insects.

The first case of the disease in man was reported in 1914 by Wherry and Lamb in the United States. From 1924 to 1937 inclusive, 8,022 cases of infection with 358 deaths were recognized (Olson 1938), though there is reason to believe that numerous other cases occurred that were not diagnosed (see Francis 1921, Culpepper 1926, Dieter 1926, Freese *et al.* 1926, Lavan 1926, MacLachlan *et al.* 1926, Parker and Francis 1926, Cumming 1930). The disease has been met with in Japan, where it is known as Ohara's disease (see Ohara 1930), in Norway (see Wefring 1930), in Soviet Russia (see Doubrowinsky 1930, Karpoff and Antonoff 1936), in Sweden (Olin 1938*a, b*), in Austria and Czechoslovakia (Drbohlav 1937), in Turkey (Arar 1937), in France (Girard 1950) and in some other countries. Cases are mainly sporadic, but occasional outbreaks have been reported. The case fatality rate is about 5 per cent.

Mode of Infection.—Infection of man is most commonly due to the handling of infected rodents, and occurs either directly through contamination of the hands with the tissues or indirectly through the bites of parasites. In the United States about 90 per cent. of cases are traced to contact with wild rabbits and hares (Cumming 1937), but in some instances infection is transmitted by wood ticks (*Dermacentor andersoni* and *D. variabilis*) and deer flies (*Chrysops discalis*) (see Francis 1921, Parker and Francis 1926, Hillman and Morgan 1937, Olson 1938).

In Norway and Sweden infection has been traced to hares (Thjotta 1930, 1931*b*, Olin 1938*b*), and in Soviet Russia to water rats (Sarchi 1929, 1930), and to sushiks (Tumansky and Kolesnikova 1935). The "lemming fever" of Norway, which follows the consumption of drinking water polluted by the bodies and excreta of the lemming, is thought to be related to tularemia (Thjotta 1931*a*). Some cases in man have been traced to contact with sheep, which may occasionally suffer from tularemia (Parker and Dade 1929, Philip and Jellison 1935). Case-to-case infection is said to be rare. The incidence of the disease is mainly in those classes of the population who are brought into contact with infected animals, such as butchers, poultry-men, and trappers. Laboratory workers are often attacked (Parker and Spencer 1926*a*). Indeed there is probably no other organism that

Physiology. The anthrax bacillus grows readily upon all the ordinary laboratory media, and growth is not improved by the addition of enriching substances. It can be grown on simple synthetic media, thiamine, magnesium, iron and calcium are required together with a source of energy, and uracil, adenine, guanine and manganese markedly stimulate growth.⁴ Growth occurs at temperatures as high as 41° to 43° C., with an optimum at 37° C. These bacillus are aerobic and facultatively anaerobic. Dextrose and trehalose are fermented rapidly but without gas production. Sucrose, maltose and some other carbohydrates are fermented less rapidly; lactose, galatose, mannitol, dulcitol, rhamnose and xylose not at all. Gelatin is slowly liquefied but indol is not formed. Nitrate is not reduced, and little or no hydrogen sulfide is produced. Milk

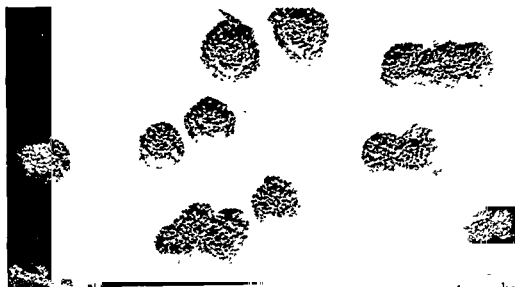


Fig. 124. Colonies of *Bacillus anthracis* on nutrient agar. Twenty-four-hour culture. Note the large size and coarse texture suggestive of R variants $\times 3$.

is feebly acidified and is curdled by a rennet-like ferment, and the casein slowly peptonized. On potato a gray, furry growth is produced; spores are formed in particular abundance on this medium.

No cultural or biochemical characteristics serve to differentiate the anthrax bacillus from the very similar nonpathogenic saprophytic sporulating bacilli, animal inoculation to determine pathogenicity is essential.⁵

The vegetative cells of the anthrax bacillus display the usual degree of resistance to deleterious influences, but the spores are relatively highly resistant, although not so resistant as the spores of *Bacillus subtilis* and related forms. Graham-Smith⁶ has found that spores exposed to daylight at room temperatures would germinate after twenty-two years but not after twenty-two and one-half years; spores dried on canvas and kept in envelopes would not germinate after thirty-five years. Anthrax spores are usually destroyed by boiling for ten minutes and by dry heat at 140° C. for three hours. Their resistance to disinfectants is variable, for 0.1 per cent mercuric chloride may fail to kill them in seventy hours, while those disinfectants which are oxidizing agents

⁴ Brewer *et al*: Arch. Biochem., 1946, 19:77.

⁵ See, for example, Stein. Amer. Jour. Vet. Res., 1944, 5 38.

⁶ Graham-Smith. Jour Hyg., 1941, 41:496.

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are much more effective, 3 per cent hydrogen peroxide kills in one hour and 4 per cent potassium permanganate in fifteen minutes. In the animal carcass vegetative cells are destroyed during anaerobic putrefactive changes in seventy-two hours, but spores are viable under such circumstances for at least nine months.⁷ In the soil anthrax spores may remain viable for many years.

Variation. The rough variant is the virulent and naturally occurring form of *B. anthracis*. It was early noted by Pasteur that prolonged cultivation of these bacilli at higher than optimum temperatures, 42.5° C., resulted in a loss of virulence and the appearance of asporogenous variants. A number of different types of variants are produced by such cultivation at high temperatures or in the presence of dilute antiseptics. Smooth and mucoid types of colonies have been observed, and some of these variants may be non-spore-forming. Virulence, however, is associated with the presence of a capsule, or the ability to form one, rather than the ability to form spores, for both asporogenous virulent strains and spore-forming avirulent strains may be found. These probably do not occur in nature, however.

Toxins. The anthrax bacillus forms neither soluble toxin nor endotoxin and its virulence appears to be associated with the glutamyl polypeptide capsule. In this connection it is of interest that the capsular substance is not hydrolyzed to an appreciable extent by the proteolytic enzymes of the body and is, in fact, excreted quantitatively by the rabbit. However, Watson *et al.*⁸ have found that an inflammatory factor, not identical with the capsular material, is produced which gives rise to tissue damage similar to that observed in infections. The activity also resembles hyaluronidase in some respects and interferes with the blood-clotting mechanism. Sterile extracts of anthrax lesions, presumably containing this activity, are capable of producing histopathological changes comparable to those of the disease.⁹ A hyperglycemia occurs during infection and in its terminal stages the disease superficially resembles magnesium poisoning, a curious observation is that the injection of calcium salts appears to have some protective effect.

The cause of death in anthrax still remains obscure. In typical anthrax septicemia bacilli are found in immense numbers clogging the capillaries and it was early supposed that death resulted from tissue anoxia due to mechanical interference with the circulation. Death may occur, however, in the absence of great numbers of bacilli in the blood and this hypothesis is not tenable.¹⁰

Pathogenicity for Lower Animals. In nature anthrax is primarily a disease of cattle and sheep, horses and swine are susceptible, but are less commonly affected. Wild deer and other gregarious herbivora are liable to occasional outbreaks. The smaller rodents are very sensitive to inoculation. Rabbits, guinea pigs and white mice are susceptible in that order, and are fatally affected by the subcutaneous introduction of a very small number of virulent bacilli. The white mouse may succumb to inoculation with a single bacillus of a highly virulent strain. Carnivorous animals, through processing

⁷ Stein, *Vet. Med.*, 1947, 42:13.

⁸ Watson *et al.*, *Jour. Inf. Dis.*, 1947, 62:121.

⁹ Gersavage, Watson, Flann and Hedley, *Jour. Inf. Dis.*, 1947, 62:14.

¹⁰ See the discussion in Flann, McGhee, Gersavage and Watson, *Jour. Inf. Dis.*, 1947, 63:107.

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greater resistance than the herbivora, are nevertheless susceptible, as several epidemics in zoological gardens involving leopards, lions, pumas, bears and other animals have shown. Certain animals possess a marked natural resistance to anthrax. Rats are quite resistant, especially the white rat, only about 14 per cent of the latter dying as the result of inoculation. The mature dog is only slightly susceptible. Birds, especially pigeons, can be infected, but not easily. Frogs are completely resistant but toads are very susceptible.

The route by which the bacilli enter the body exerts an important influence in both experimental and natural infections. Subcutaneous inoculation is the method most commonly practiced in experimental work, and is almost uniformly fatal with the ordinary small laboratory animals. Feeding experiments show that administration of spore-free cultures even to highly susceptible animals is without result, owing to the destruction of the bacilli in the stomach. The feeding of spores, on the other hand, leads to infection of the more susceptible species, although not so certainly as subcutaneous inoculation; resistant species, such as swine, may be infected through the alimentary tract only with difficulty. Infection through the respiratory tract is possible in the experimental animal but is probably almost unknown in the lower animals under natural conditions.

In highly susceptible animals the disease is acute and runs a rapid course, the case fatality in cattle and sheep is about 80 per cent. It presents all the characteristics of a typical septicemia and local manifestations may be almost entirely absent. Enormous multiplication of the bacteria takes place in the blood and internal organs, and sections through the liver or spleen show the capillaries gorged with masses of bacteria. The spleen is of a deep-red color and greatly enlarged, hence the name splenic fever. The more resistant animal species do not develop this generalized infection, but the bacteria remain localized in an abscess or carbuncle and fail to spread through the body. This is the case with the dog and in some forms of infection in man. In this respect anthrax furnishes an illustration of the general rule that when a bacterial invasion meets slight resistance from the animal tissues an abundant multiplication of the bacteria occurs throughout the body, while the possession of high powers of resistance is accompanied by a pronounced local reaction. Man stands perhaps midway in susceptibility between the dog and the sheep.

Under natural conditions cattle and sheep are infected through the alimentary tract by swallowing spores while grazing in infected pastures. As has been pointed out, spores are able to retain their vitality in soil for a long period, and pastures once infected may infect cattle after a lapse of as many as thirty years. Hides imported from China and other countries where the disease prevails are not uncommonly contaminated with anthrax spores, in the United States several outbreaks of anthrax among cattle with some consequent cases of human infection have been traced to the overflowing of pasture land by streams receiving the drainage of tanneries.

Cattle may also occasionally be infected by direct contact through wounds, abrasions and other injuries to the skin; but alimentary infection is by far the most common. Anthrax has been experimentally transmitted to susceptible animals by biting flies of various species that had previously fed on animals

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dying from anthrax.¹¹ The bacilli persist in the insects for only a short time

Pathogenicity for Man. Three routes of infection of human beings are known: (a) through the skin, (b) through the respiratory tract and (c) through the alimentary tract. The bacillus is almost always transmitted to man through the agency of the lower animals rather than through other human beings. The persons most commonly affected are those having to do with cattle and their products, such as butchers, shepherds and herdsmen, handlers of hides, hair and fleeces. In the United States there were 357 cases of anthrax and 52 deaths in 1934-38, and in 1939-43 408 cases and 33 deaths.¹² The incidence of cases of infection from wool and hair increased nearly five-fold in the second five year period and over 90 per cent of the infections contracted in tanneries were from goat skins. During the first World War less efficient preliminary disinfection of hides and bristles permitted the introduction of anthrax-contaminated articles from parts of Asia and South America, and a striking increase in anthrax occurred from the use of shaving brushes—the bacilli were isolated from brushes purchased in the open market in some instances. The bacilli are destroyed on such brushes by soaking in 10 per cent formalin at 110° F. for four hours. Laboratory infections, sometimes fatal, have been known to occur with pure cultures of the anthrax bacillus. The case fatality of anthrax in man is probably about 20 per cent.

Cutaneous Anthrax (Malignant Pustule) The most common form of anthrax in the human subject is due to skin infection, and usually takes the form of a localized boil or abscess, which often heals spontaneously but may progress into a septicemic condition unless checked by incision or other surgical procedure. Owing to the relatively high resistance of man septicemia does not often occur, especially if the carbuncle be incised and thoroughly drained. Lesions of all sizes may be produced, from a minute pustule to a large abscess.¹³

Pulmonary Anthrax. The pulmonary form of anthrax due to inhalation of the microorganisms is the most dangerous, although not the most common, variety of the disease in man. It is an occupational disease among those who handle and sort wools and fleeces and contract the infection by inhalation of spores set floating in the air from the infected material, pulmonary anthrax is known in England as "wool-sorters' disease." It is characterized by many of the symptoms of pneumonia and often passes into a fatal septicemia. Experimental air-borne anthrax was studied intensively by Young, Zelle and Lincoln¹⁴ and it was found that a very few spores sufficed in the case of virulent strains, entering the tissues from the alveoli via the lymphatic system. The inhaled spores produced only slight local reaction except for a clogging of the capillaries in the terminal stage of the disease.

Intestinal Anthrax. The alimentary tract, although the usual path of infection in cattle, is very rarely so in man. A few instances are on record of

¹¹ Mitzman. Hyg. Lab. Bull. No. 94, 1914. See also Kraneveld and Djaenoeidin Nederland. Indische Blad. Diergeneesk., 1940, 52-339.

¹² Report, Committee on Industrial Anthrax. Amer. Jour. Pub. Health, 1945, 35-850.

¹³ For a discussion of cutaneous anthrax see Hodgson. Lancet, 1941, ii-811, Gold Arch. Int. Med., 1942, 70-785, Ellington, Kadull, Baskwalter and Howe. Jour. Amer. Med. Assn. 1946, 131-1105.

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intestinal anthrax contracted through the medium of spore-infected food. Such cases occur among workers with animal products, and have probably been due to lack of caution in handling food with uncleansed hands. Insufficiently cooked meat from anthrax-infected animals may also be a source of intestinal anthrax.

Immunity. The cause of the high natural immunity to anthrax possessed by the dog, the fowl and certain other animals has been the object of much experimentation, but no clear-cut explanation has as yet been found. The body fluids of some species manifest bactericidal powers toward the anthrax bacillus, but there is no concurrence between the degree of immunity and the anthracidal power of the blood serum. The blood serum of the highly susceptible rabbit is strongly bactericidal outside the body, but anthrax bacilli injected into the circulation seem to multiply freely in the blood stream. Blood taken

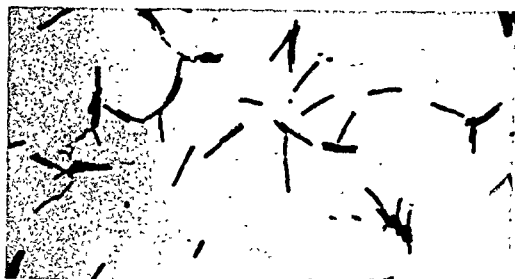


Fig. 125. *Bacillus subtilis*, twenty-four-hour culture on nutrient agar. Crystal violet stain. No spores have formed as yet. Note the typical arrangement of the bacilli $\times 1200$.

from the very resistant dog and fowl is practically devoid of bactericidal properties.

Acquired immunity to anthrax is a consequence of the presence of living, virulent bacilli in the body; suspensions of killed cells or completely avirulent bacilli do not produce an immunity. Pasteur devised a method for vaccinating cattle and sheep against anthrax which is dependent on the subcutaneous inoculation of attenuated cultures. Two vaccines were used. The "first vaccine" consisted of bacilli grown at 42.5°C . for fifteen to twenty days and would not always kill guinea pigs though still fatal for white mice. After twelve days a second inoculation was made with the "second vaccine" of bacilli cultivated at the higher temperature ten to twelve days, which would kill guinea pigs but not rabbits. Following inoculation with these two vaccines, a fully virulent culture could be injected with impunity. In spite of some accidents due to the use of imperfectly standardized vaccines, this method of protective inoculation has proved, on the whole, of great practical value. In France 30,000 to 50,000 cattle and horses and 250,000 to 350,000 sheep are vaccinated

CHAPTER 76

ANTHRAX

HISTORY

ANTHRAX is a disease that has been known from antiquity. In earlier days, however, it was not clearly separated from other affections closely simulating it. Maret (1752) and Fournier (1769) defined the clinical type of malignant pustule in man, and Chabert (1780) gave a clear description of anthrax in animals. In 1823 Barthelémy showed that it was transmissible by inoculation. Rayer (1850) described small, non-motile, filiform bodies in the blood of sheep dead of the disease, and confirmed its transmissibility by inoculation. (For references see Hutyrá and Marek 1922.) In a series of papers, Davaine (1863*a*, *b*, *c*, 1864) showed that anthrax could be transmitted to sheep, horses, cattle, guinea-pigs, and mice, by the subcutaneous inoculation of infected but not of normal blood; that in such animals the bacilli did not appear in the blood till 4 or 5 hours before death; that in the blood they increased rapidly in numbers, and became filamentous; and that after death they disappeared as soon as putrefaction commenced. He showed, moreover, that the blood of an infected animal, previous to its invasion with the bacilli, was non-infective, but that after invasion it was capable of conveying the disease; that animals fed on infected viscera frequently became infected, but that animals fed on the putrefying organs of non-infected animals did not do so; and that after death from anthrax the spleen, liver, kidneys, lungs, blood, and, to a less extent, other organs contained the bacilli in large numbers. In the same year, Tiegel and Klebs (see Koch 1881) showed that anthrax blood, if filtered through a clay candle, was deprived of its infectivity; the filtrate was innocuous to animals, but the deposit on the filter remained active. These observations showed, as conclusively as could be expected in the absence of cultivation, that anthrax was caused by a living organism that multiplied in the body, invaded the blood stream, and produced death by septicæmia. To this organism Davaine gave the name of *Bactéridie*—a name by which it is still known among French writers. Subsequently Davaine and Raimbert (1864) found the same organism in a malignant pustule in man, thus demonstrating the ætiological identity of the disease in man and animals.

The final proof of the causative rôle of *B. anthracis* was produced by Koch (1877), who, in a classic masterpiece which brought him suddenly into fame, gave a full account of the organism, described its formation of resistant spores, its cultivation *in vitro*, the reproduction of the disease by injection of pure cultures, and the recovery of the organism from the animals at necropsy.

The subsequent history of anthrax is largely connected with attempts at

annually. Active immunization of rabbits and guinea pigs can also be effected by the injection of attenuated cultures, but with much greater difficulty.

The simultaneous inoculation of anti-anthrax serum and a spore vaccine (Sobernheim's method) has been quite extensively practiced in the United States in districts where anthrax is prevalent, and here, too, occasional infections with the vaccine occur. It has been found by Cromartie *et al.*¹⁵ that the sterile extract from anthrax lesions which produces histopathological changes comparable to those found in the disease is an effective immunizing agent in experimental animals. The immunizing agent is, however, distinct from the inflammatory factor. It was early postulated by Ascoli that immunity to anthrax involves some process which retards capsule formation, and the evidence of

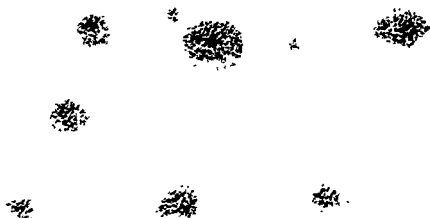


Fig. 126. Colonies of *Bacillus subtilis* on nutrient agar. Twenty four hour culture. Note the resemblance to colonies of the anthrax bacillus $\times 3$.

recent years is in accord with this hypothesis, but very little is known of the mechanisms involved.

The serum of actively immunized animals contains specific protective substances, and inoculation with such antisera confers some degree of passive immunity. Antisera have been used in the treatment of human anthrax with encouraging results. At the present time it is customary to treat anthrax cases with a combination of antiserum and arsenicals,¹⁶ and recent evidence suggests that penicillin is of value.

Bacteriological Diagnosis of Anthrax. If the specimen is fresh and not grossly contaminated the anthrax bacillus may be found in gram-stained smears as a rule and is readily cultured on the usual nutrient media. Such a specimen may also be used for guinea pig inoculation, but it must be borne in mind that the sporulating obligate anaerobes will kill as rapidly as the anthrax bacillus. In any case the isolated culture must be tested for pathogenicity by

¹⁵ Cromartie, Watson, Bloom and Heckly: Jour. Inf. Dis., 1947, 80 14.

¹⁶ Cf. Amer. Pub. Health Assn. Yearbook, Suppl. Amer. Jour. Pub. Health, 1935, 25 No. 2 see also Cruckshank: Lancet, 1939, ii 681.

animal to animal. Infection generally occurs by the alimentary tract from ingestion of infected food. During the last stages of the disease in animals, the bacilli are excreted in the urine, faeces, and saliva. At the time of death and for some time afterwards, bloody infected fluid exudes from the openings of the body, and soils the neighbouring ground. The bacilli, which in the blood are invariably in the vegetative form, after being voided from the body soon produce spores under the influence of a suitable temperature and free access of oxygen. These spores are extremely resistant to inimical agencies and may remain alive on the surface of the ground for as long as 12 years (Pasteur 1881a). Cattle or sheep feeding on this ground are liable to be infected; the spores are taken in by the mouth; they probably pass through the stomach unharmed, multiply in the small intestine, invade the mucosa, and reach the blood stream.

This view, which was largely developed by Koch (1881) differed in some respects from that put forward by Pasteur (1880), who maintained that infection occurred through the mouth and pharynx. As evidence of this, he showed that if sheep were fed on spores, a certain proportion died of anthrax; but that if to the infected food were added prickly substances, such as the pointed extremities of dried thistle leaves or barley spikes, which were able to cause erosions of the pharyngeal mucosa, the mortality amongst the sheep was increased. Pasteur, moreover, maintained that earthworms played an important part in the actual contamination of the ground. He supposed that, when an animal was buried, the bacilli exuding from the openings of the body developed into spores, which were then brought by earthworms to the surface of the soil, where they were deposited in casts. It is doubtful what importance is to be attributed to this view. Koch (1881), who examined a number of earthworms placed in artificially infected soil, found that they rarely became infected; and he brought indirect evidence to suggest that their rôle in the contamination of the ground was negligible in comparison with that caused by the exudation of infected body fluids from animals just before or after death. It is probable that both birds and carnivora, by carrying portions of imperfectly disposed animal carcasses, can transmit infection from one area to another (Cameron 1945).

The view that the infection of animals occurs chiefly by feeding on contaminated pasture land affords a possible explanation of the greater prevalence of anthrax in low-lying, marshy areas, and by the banks of streams, where grasses and decaying vegetable materials are abundant on which the bacillus may grow; of the rise in incidence as soon as the weather becomes warm, and of the absence of an æstival prevalence of the disease in cold countries. It is, however, by no means clear that the anthrax bacillus can multiply on the surface of the soil under natural conditions, and the view is held by some workers that the organism is an obligatory parasite (see Minnett and Dhanda 1911). The contamination of the soil may be due, not to multiplication of the organisms outside the body, but to the favourable effect of a high temperature on sporulation. Minnett (1950), for example, found that in an open carcass spore formation occurred fairly rapidly in blood at 90° F., but that at 60-70° F. the bacilli disintegrated owing to the growth of contaminants before spore formation could occur.

In Great Britain, as already stated, the incidence is highest during the winter months. This fact alone is sufficient to suggest that the mode of infection is different from that in warmer climates. Though occasional infection may occur by contamination of the ground with the effluents from tannery and other industrial works, M'Fadyean (1903b) brought evidence to show that soil infection plays little part in the spread of the disease in this country; and that the majority of cases

animal inoculation. The animal will die in thirty-six to forty-eight hours after subcutaneous inoculation of a very small amount of culture. A gelatinous infiltration will be found at the site of inoculation and the tissues of the animal contain enormous numbers of the bacilli; smear of cut spleen, for example, will show many of the large gram-positive bacilli. This demonstration of pathogenicity is sufficient for identification since none of the aerobic sporulating rods that resemble the anthrax bacillus is pathogenic for the guinea pig and similar experimental animals.

A precipitation test, the Ascoli test or thermo-precipitation test, is sometimes used to detect anthrax contamination of hides or other tissues. The specimen is



Fig. 127. Colonies of *Bacillus mycoides* on nutrient agar. Twenty-four-hour culture. $\times 3$.

extracted with boiling water and the extract used as an antigen in a precipitation ring test with very high titer anthrax antiserum.

RELATED BACILLI

As indicated earlier, there are many species of aerobic sporulating bacilli closely related to and indistinguishable from the anthrax bacillus on any basis other than pathogenicity. The close morphological similarity led many of the early workers to describe "avir" and "Bacillus anthracoides." On the contrary, the vast majority of these bacilli are saprophytic forms and are commonly found as contaminants on plates because of the wide distribution of their spores in dust.

Of the commonly encountered forms, *B. subtilis*, *B. megatherium* and *B. cereus* are among the most familiar. *B. mycoides*, sometimes called *B. ramosus*, is closely related to *B. cereus* and is classified by Bergey (1948) as a variety of it rather than a separate species. There are some 33 species of *Bacillus* in all. The following portion of a key to the genus is abridged from Bergey (1948) and indicates the manner in which species are separated and defined. The close relation of the anthrax bacillus to the saprophytic forms is noteworthy.

The second type of anthrax, which may take the form of *malignant pustule* or pulmonary disease, is dependent on infection acquired during the industrial treatment of animal products, and shows no special seasonal incidence. For convenience of reporting, the cases in this country are divided into 5 groups (Table 154)

Anthrax is not a common disease in either Great Britain or the United States of America. The number of cases notified under the Factories Act 1895 in England and Wales during the 20 years 1932-51 was 492, of which 46 were fatal. Records are not available for the number of non-industrial cases, but 35 deaths occurred, making a total of 81. In the United States during the 31 years 1919-49 the total number of cases was 2,447; of these, approximately two-thirds were of industrial and one-third of agricultural origin (Wolff and Heimann 1951).

At one time anthrax was not uncommon in the woollen industries of the West Riding of Yorkshire and of Worcestershire, where the dangerous classes of raw wool from Asia Minor and Persia were used, but it has now been largely brought under control. In the horsehair industry those using contaminated hair from China, Russia, and Siberia are chiefly affected. Among the dock porters anthrax is seen mainly in the ports of London and Liverpool. (For incidence of human anthrax in Germany, Austria, and the U.S.A., see Report 1934-5, Wolff and Heimann 1951, Steele and Helvig 1953)

TABLE 154
INCIDENCE OF INDUSTRIAL ANTHRAX IN GREAT BRITAIN *

Year.	Wool.	Horsehair.	Hides and Skins.	Other Industries.	Dock Labourers.	Total.
1913 . . .	43 ⁽⁵⁾	5 ⁽¹⁾	19 ⁽²⁾	3	3	73 ⁽⁸⁾
1914 . . .	29 ⁽⁵⁾	5	15	6 ⁽¹⁾	7 ⁽²⁾	62 ⁽⁸⁾
1915 . . .	26 ⁽²⁾	2	18 ⁽³⁾	3 ⁽¹⁾	0	49 ⁽⁵⁾
1916 . . .	80 ⁽¹²⁾	6 ⁽³⁾	18 ⁽³⁾	2	2 ⁽¹⁾	108 ⁽¹⁸⁾
1917 . . .	65 ⁽¹¹⁾	1	20 ⁽²⁾	4 ⁽¹⁾	3 ⁽¹⁾	102 ⁽¹⁵⁾
1918 . . .	40 ⁽⁴⁾	4 ⁽²⁾	14 ⁽¹⁾	1	3	71 ⁽⁷⁾
1919 . . .	34 ⁽⁵⁾	3 ⁽¹⁾	16 ⁽¹⁾	4 ⁽²⁾	3 ⁽²⁾	60 ⁽¹¹⁾
1920 . . .	25 ⁽⁷⁾	5 ⁽¹⁾	17 ⁽³⁾	1	4 ⁽¹⁾	52 ⁽¹²⁾
1921 . . .	11 ⁽³⁾	4 ⁽¹⁾	8 ⁽¹⁾	2 ⁽¹⁾	0	25 ⁽⁵⁾
1922 . . .	19 ⁽³⁾	9 ⁽¹⁾	16 ⁽¹⁾	1	1	46 ⁽⁵⁾
1923 . . .	14 ⁽¹⁾	9 ⁽²⁾	22 ⁽¹⁾	1 ⁽¹⁾	4 ⁽³⁾	50 ⁽⁸⁾
1924 . . .	19 ⁽¹⁾	4 ⁽¹⁾	16 ⁽²⁾	4	2	45 ⁽⁴⁾
Total . . .	414 ⁽⁵⁹⁾	57 ⁽¹³⁾	208 ⁽³⁰⁾	32 ⁽⁷⁾	32 ⁽¹⁰⁾	743 ⁽¹⁰⁰⁾

The figures in brackets indicate deaths.

* For this Table, we are indebted to the kindness of Dr. S. A. Henry, H.M. Medical Inspector of Factories.

In all but the woollen industry, the type of anthrax contracted is the malignant pustule. As infection occurs from contamination of the skin with the infected animal products, it is natural to expect that the uncovered parts of the body will suffer most severely. Legge (1934) gives the following table (Table 155), showing the site of the pustule.

The site of the lesion varies, moreover, with the nature of the industry. Hide porters, for example, are frequently infected on the back of the neck, which is more open than other parts to excoriation. In butchers and others who have to handle

Mesophilic, aerobic bacilli with spores ellipsoidal to cylindrical and central to terminal

- (I) Diameter of vegetative cells less than 0.9μ (small cell variety)
 - (1) Grow at pH 6.0, acetylmethylcarbinol formed
 - (a) Gelatin hydrolyzed
 - (i) Starch hydrolyzed, nitrate reduced to nitrite
Bacillus subtilis
 - (ii) Starch not hydrolyzed, nitrate not reduced to nitrite
Bacillus pumilus
 - (b) Gelatin not hydrolyzed
Bacillus coagulans
 - (2) No growth at pH 6.0, acetylmethylcarbinol not formed
 - (a) Casein digested, urease not formed
Bacillus firmus
 - (b) Casein not digested, urease formed
Bacillus lentus
- (II) Diameter of vegetative cells 0.9μ or more (large cell variety)
 - (1) Acetylmethylcarbinol not produced
Bacillus megatherium
 - (2) Acetylmethylcarbinol produced
 - (a) Saprophytic, usually motile
Bacillus cereus
Bacillus cereus var. *mycoides*
 - (b) Pathogenic, non motile
Bacillus anthracis

These forms can be distinguished from one another on the basis of details of spore formation, differential fermentations and the like, and constitute stable types. Immunological investigation has confirmed the homogeneity of these types also. The immunological specificity of the spores appears to be different from that of the cell substance, and Lamanna¹⁷ has found that four main types of the small cell group can be distinguished though differentiation of the large cell group by this means is not so satisfactory. Sievers¹⁸ has reported similar results in studies of the specificity of the cell substance.

The pathogenicity of these forms is very slight at best but *B. subtilis* is occasionally responsible for infection, particularly of the eye, and rarely may produce a septicemia in the immature animal. Other bacteria of this group are occasionally found to have feeble pathogenic powers. Heaslip¹⁹ isolated an aerobic sporulating bacillus which he called *Bacillus tropicus* by the inoculation of mice with blood from persons suffering from a mild infection in Australia called "coastal fever." This bacillus has also been found there as a natural parasite of the rat and the bandicoot. It appears to be very similar to a bacillus described by Scott many years before as *Bacillus seroficus*. *Bacillus alvei*, the cause of fowlbrood of bees, is not pathogenic for man.

¹⁷ Lamanna Jour. Inf. Dis., 1940, 67:193, 203.

¹⁸ Sievers Jour. Bact., 1942, 43:305.

¹⁹ Heaslip Med. Jour. Australia, 1941, 2:536.

whenever a case occurs in an industrial establishment, anthrax bacilli can be found, often in large numbers, widely distributed in the environment.

Apart from malignant pustule, and the less common respiratory and intestinal forms of the disease in man, meningitis is occasionally caused by the anthrax bacillus.

Bacteriology of Anthrax in Man and Animals.

Anthrax is a disease that, when fatal, invariably terminates in septicæmia. Whatever form the disease takes—the malignant pustule, respiratory, or intestinal—it is characterized by a primary local proliferation of the bacilli, with the formation of local lesions. The fate of the animal rests on the result of this local attack; if it is resisted by the phagocytes and other defences of the body, recovery rapidly occurs; if the bacilli prove too virulent, they invade the blood stream, and multiply abundantly. Invasion occurs late—generally not more than a few hours before death—and is accompanied by severe toxic manifestations. It is characteristic of anthrax that the bacilli remain confined almost entirely to the blood vessels; they are found in maximum numbers in the capillaries of the liver, lung, kidney, spleen, intestine, and stomach, in smaller numbers in those of the brain, skin and muscle. Their distribution varies, however, with the animal attacked. In the blood of mice or rabbits, for example, there are few bacilli to be found; in guinea-pigs there may be more bacilli than red blood corpuscles. In the larger animals they are usually plentiful, but pigs and horses may die before the organisms have proliferated sufficiently to be detected microscopically (Stockman 1911).

In man, the malignant pustule starts as a small area of inflammation. It increases in size at a varying rate. Coagulation necrosis of the centre occurs, leading to the formation of a brown, purplish or black eschar surrounded by an intermediate zone of vesicles filled with clear yellow or sanious fluid, and an outer zone of widespread cedema and induration. There is no true pus and very little pain (Hodgson 1941). The bacilli are most abundant immediately below the central necrotic area. Invasion of the blood stream may occur, but the number of organisms is seldom as great as in the larger animals (Eurich 1930).

Experimental Production of Anthrax in the Larger Animals.—Under natural conditions, the disease is confined chiefly to the herbivora and to man, but occasionally other animals are attacked. Experimentally the disease can be reproduced in the herbivora, rodents, and omnivora; birds, with the exception of sparrows, and to a less extent hens, ducks, and pigeons, are resistant. Reptiles and fish are insusceptible to infection.

Most of the early experiments on the larger animals were performed with the infected blood of other animals, and it was found that for transmission to be successful certain precautions had to be taken. Putrid blood, for example, often proved non-infective, as did blood that had been rapidly dried after removal from the body. On the other hand, blood or tissues that had been dried slowly were found to retain their infectivity for at least 4 years (Koch 1877). The occurrence of these anomalies was shown by Koch (1877) to be dependent on the presence or absence of spore formation. He found that whenever the bacilli had formed spores, and these spores were capable of being cultivated *in vitro*, the material containing them was infective to animals. The blood of a foetus removed from a cow that had succumbed to anthrax proved innocuous, showing that the organisms had not passed the placental filter.

The disease produced in the larger animals is similar to that occurring naturally.

CLOSTRIDIUM—THE SPORE-FORMING ANAEROBES¹

The group of anaerobic sporulating bacilli includes a variety of forms. Some of these, the anaerobic nitrogen-fixing bacteria, the butyl alcohol and acetone-producing forms and others, have been discussed earlier (Chap. 4). Still others, however, are pathogenic for man and lower animals, and the more important of these forms—*Clostridium tetani*, *Clostridium septicum*, *Clostridium welchii*, *Clostridium novyi*, *Clostridium histolyticum*, *Clostridium chauvei*, *Clostridium botulinum* and the non-pathogenic but common species *Clostridium sporogenes*—will be considered here.

The status of these bacteria as parasites is open to some question. They occur in the soil, in particular abundance in manured soils, and are found in the intestinal tract of man and animals. *Cl. welchii*, for example, is uniformly present in human feces, and the tetanus bacillus is often found (in up to 40 per cent of specimens examined) in the feces of domestic animals. It has been assumed by some that these bacilli are parasitic and their presence in the soil is a consequence of contamination. Although their numbers are unquestionably greatly increased by manuring and other forms of contamination, some have been found in virgin soils. It is perhaps best to regard them as essentially saprophytic soil forms that are capable of maintaining themselves in the large intestine.

None of these bacteria possesses any marked ability to invade the body tissues by itself. *Cl. botulinum* is apparently incapable of setting up an infection, while others, such as the tetanus bacillus, produce local infections when aided by traumatic injury to the tissues and frequently by the presence of other bacteria. Still others, such as the bacilli associated with gas gangrene, show pronounced invasive properties when once established, but the initial invasion is made possible by other factors, usually trauma and the presence of other bacteria.

The pathogenicity of the anaerobic bacilli is, rather, attributable to their ability to form powerful exotoxins, a property which is curiously confined to these bacteria, the diphtheria bacillus and possibly the Shiga dysentery bacillus. In the case of botulism the toxin is preformed outside the animal body and, since it is unique in that it is resistant to the digestive enzymes, enters the body by way of the alimentary tract and absorption into the tissues. In the other cases a focus of infection is established and the toxin formed at that point is disseminated through the body. In some instances, such as gangrene,

¹ These and other anaerobic bacteria are considered at length by Weinberg, Nativelle and Prevot: *Les Microbes Anaerobies*, Masson et Cie., Paris, 1937. The serological relationships of these bacteria are reviewed by McCoy and McClung *Bact. Rev.*, 1938, 2, 47.

welchii, a large Gram-positive capsulated anaerobic bacillus which may invade the blood stream under certain conditions; the exclusion of this organism must rest on cultural examination. Their number varies greatly. In the fluid taken from a malignant pustule in man, they may be so few as to escape observation; but if the base of the vesicle is

scraped gently with the back of a scalpel and films are prepared with the scrapings, or if sections are made of a portion of excised tissue, embedded in paraffin and stained by Gram, the bacilli can generally be found. Not too much attention should be paid to negative findings.

Cultural Examination.—

Where possible, fresh material should be used. The blood or tissue juice may be taken on a swab, a sterile thread, a fragment of earthenware, or a piece of gypsum that has been soaked in broth and subsequently sterilized (Strassburg method). The drying of the material prevents the destruction of the bacilli by the bactericidal power of the serum (Eurich 1933), facilitates their rapid sporing, and

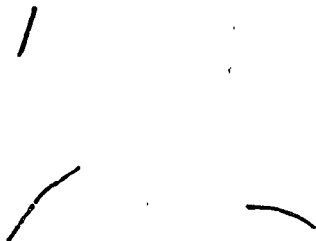


FIG. 291.—*Bacillus anthracis*.

Smear from a malignant pustule in man, showing the bacilli in small numbers ($\times 1000$).

prevents the growth of other organisms. Cultures are put up in the usual way on to agar plates and into broth, and the resulting growth is carefully studied. It is important not to confuse *B. anthracis* with other members of the aerobic spore-bearing group. To avoid this, it should be remembered that the anthrax bacillus is non-motile, and liquefies gelatin slowly, whereas most of the other members are motile, and liquefy gelatin rapidly. (For the further differentiation of *B. anthracis* from anthrax-like bacilli, see Chapter 35.)

If wool or hair is to be examined, it should be soaked in water or a weak solution of KOH for about 4 hours and then squeezed or teased out. The supernatant fluid should be withdrawn, heated to 70° C. for 10 minutes, and distributed in quantities varying from 0.02 to 2 ml. into pour plates of 1–2 per cent. peptone agar. After 16 hours' incubation the plates should be examined for deep colonies, which often have a characteristic opaque white filamentous appearance with sometimes a nebula at one end (Eurich 1912). Suspicious colonies should be subcultured into the inner tube of a Craigie tube. The anthrax bacillus grows both on the surface and in the depth of the medium without showing any evidence of motility. Further confirmation can be obtained by examining the growth on egg-yolk medium on which the anthrax bacillus gives a weakly positive lecithinase reaction, by testing its susceptibility to the specific bacteriophage of McCloy (1951), and by inoculation into guinea-pigs.

Pearce and Powell (1951) described a selective medium for the suppression of soil bacteria. It makes use of the fact that the anthrax bacillus is not inhibited by the presence of 50 μ gm of haematin per ml. in nutrient agar or by lysozyme. On this medium, particularly if incubated at 39–40° C., less than 5 per cent. of aerobic spore-bearers in soil,

an extensive local destruction of tissue occurs, but in general the diseases caused by these bacilli are essentially toxemias

It will be clear from the foregoing considerations that infections with the sporulating anaerobes are not common under ordinary circumstances, for in most instances traumatic injury is a preliminary to infection. On the battlefield, however, such injuries are common, and tetanus and gangrene are not infrequent complications of war wounds. Such anaerobic wound infections were prominent in the first World War, perhaps as a consequence of battles fought over the heavily manured fields of France. The occurrence of gaseous gangrene during the World War II among troops in North Africa where the desert soil is relatively free of such forms suggests that clothing may be a more important source of contamination than had been supposed.

Cl. histolyticum is microaerophilic, i.e., it will grow in the presence of small amounts of oxygen, but the remainder of the forms considered here

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE MORE IMPORTANT PATHOGENIC ANAEROBES

	Spores	Cap- sule	Motil- ity	Prote- olysis	Fermentations			
					Dev- trose	Lac- tose	Su- crose	Exo- toxin
<i>Cl. tetani</i>	spherical, terminal	—	+	—	—	—	—	+++‡
<i>Cl. septicum</i>	oval, sub terminal	—	+	+sl*	+	+	—	++
<i>Cl. welchii</i>		+	—	+sl	+	+	+	++
<i>Cl. novyi</i>		—	+	+sl	+	—	—	+++
<i>Cl. histolyticum</i>		—	+	+	a†	—	—	+
<i>Cl. sporogenes</i>		—	+	+	+	—	—	—
<i>Cl. chauveti</i>		—	+	+sl	+	+	+	++
<i>Cl. botulinum</i>		—	+	±	+	±	—	+++

*sl relatively slight, † a acid, ‡ strong, moderate and weak

are obligate anaerobes. They may be isolated in pure culture by picking colonies from a shake culture or from plates incubated in an anaerobic jar. All are large gram positive rods, non-encapsulated with the exception of *Cl. welchii*, and motile by means of peritrichous flagella with the exception of *Cl. welchii*. Spores are usually of a greater diameter than the vegetative cells, and the spore-containing cells are spindle- or club-shaped. The spores of the tetanus bacillus are round and terminal, and those of the other bacilli oval and subterminal. Two general physiological types of anaerobic bacilli may be distinguished, the one predominantly fermentative or saccharolytic and the other predominantly proteolytic. These and other characteristics

are produced in the fermentation. Ter in this respect from the other pathogenic bacteria which produce non-volatile acid, i.e., lactic acid, for

probably be better to use a serum containing precipitins for both components (see also Ivánovics 1939).

Natural Immunity.

The investigation of the natural resistance of certain animals to anthrax played a prominent part in the controversy between the cellular and humoral schools of immunity in the early days of bacteriology. Without attempting to adduce all the evidence that was brought forward during this controversy, we shall merely draw attention to some of the salient points. Von Behring (for references see Chapter 52) found that the blood serum of the rat—an animal that is fairly resistant to anthrax—possessed a remarkable destructive action on the bacillus and he was led to conclude that the immunity of this animal was dependent on the bactericidal content of its serum (see p. 1344). On the other hand, Metchnikoff and his co-workers stated that, though the blood serum of the rat is bactericidal *in vitro* to the anthrax bacillus, the blood plasma *in vivo* does not possess this action. Furthermore, the spores are able to develop in the body of the rat, and to cause a symptomless infection (Kritschewski and Messik 1930); their further proliferation was said to be restrained by the attack of the phagocytes, which ingested and destroyed them. The serum of the rabbit, like that of the rat, has a bactericidal effect on the anthrax bacillus, yet the rabbit is susceptible to experimental inoculation with this organism.

The dog presents another interesting example. When young it is fairly susceptible; when older it is more resistant to experimental infection. Nuttall (1888) found that the defibrinated blood of the dog readily destroyed the bacilli; Metchnikoff was unable to confirm this, but demonstrated a close correlation between the phagocytic activity of the leucocytes and the resistance of the animal. Methods such as removal of the spleen, or intravenous injection of fine wood charcoal, which served to divert the leucocytes, destroyed this resistance.

Pasteur, Joubert and Chamberland (1878) found that the natural resistance of fowls to infection with anthrax could be lowered by immersing them up to the thighs in cold water; they concluded that the immunity of these birds depended on their high normal temperature (41–42° C.), which interfered with the development of the bacillus. That this explanation was incorrect was shown by Wagner (1890), who found that the bacillus developed readily in the blood and blood serum of fowls outside the body, even at a temperature of 43° C. Further experiments led to the conclusion that the immersion in cold water served to lower the phagocytic response, and thus allow the bacilli to proliferate. We may also quote the experiments of Charrin and Roger (1890), who stated that the natural resistance of rats could be broken down by excessive exercise, and of Preisz (1909), who found that frogs were resistant if kept at 18° C. but not at 30° C.

Weyl (1892) found that anthrax-infected threads, planted in the subcutaneous tissues of hens or pigeons, were rendered avirulent for mice in 4 days; he satisfied himself that the bacilli were killed by the phagocytes as soon as the spores germinated.

Chauveau (1880c) stated that, if anthrax bacilli were injected intravenously into vaccinated animals, the bacilli rapidly disappeared from the circulation, being filtered off by the lungs and spleen; these bacilli, however, retained their vitality for some days. Sobernheim (1904) likewise found that the bacilli could live for weeks in the local infiltration produced in immunized sheep without undergoing any loss of virulence. From these experiments, it is clear that a number of bacilli may remain alive and virulent in the tissues without giving rise to disease.

From these early observations it is difficult to draw any firm conclusions on

the most part. Amino acids are vigorously attacked by the obligate anaerobes; some are "fermented" to organic acids,² while others are mutually oxidized and reduced, a process that has been discussed elsewhere (p. 106).

TETANUS (CLOSTRIDIUM TETANI)

Tetanus is a disease of man and animals characterized by spasms of the voluntary muscles. The spasms are often most marked in the muscles of the jaw and neck, hence the name "lockjaw." The tetanus bacillus was first described in 1884 by Nicolaier, who observed it in the pus taken from mice and other animals that had died after subcutaneous inoculation with small quantities of soil. Kitasato isolated the microorganism in pure culture in 1889 and demonstrated its causal significance. He also proved the inability of the tetanus bacillus to invade the blood stream and showed the disease to be

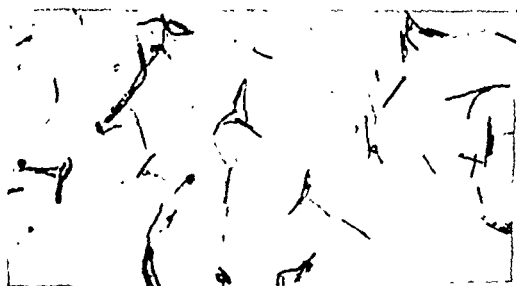


Fig. 128. *Clostridium tetani* in pure culture. Young, actively growing culture showing beginning spore formation. Note the refractile, unstained spores, the drumstick appearance when these are attached to the cells, and the tendency of the vegetative cells to remain attached end to end. Fuchsin, $\times 1150$.

an intoxication. In 1890 von Behring and Kitasato laid the basis for antitoxic therapy in their discovery of diphtheria and tetanus antitoxins.

Morphology. Individual tetanus bacilli are slender, motile (20 to 30 peritrichous flagella), gram-positive, sporulating rods with rounded ends. Their common dimensions are 0.3 to 0.5 μ in width and 2 to 5 μ in length, but vegetative filaments of much greater length occur. The shorter forms are usually straight, filaments tend to curve in an undulating manner. Short chains of rods may occur. The spore is spherical and terminal and larger in diameter than the vegetative cell, spore-containing cells have a characteristic drumstick appearance (Fig. 128). Isolated colonies in deep dextrose agar have a woolly appearance and may either be flocculent or have an opaque center. Surface colonies are flat, rhizoid, or even feathery, and frequently exceed 1

² Such as the conversion of glutamic acid to butyric and acetic acids, carbon dioxide, ammonia and hydrogen. Cf. Barker. Jour. Bact., 1938, 36 322.

of the imported animal products, wool, hides, horsehair, and so on, and partly to diminishing the risk of contact with dangerous material in the factories. For the various methods of disinfection, and for factory legislation, the reader is referred to textbooks of Hygiene. Briefly, however, the disinfection of bales and fleeces may be accomplished by preliminary treatment with warm alkali and soap, followed by exposure to a 2 per cent. solution of formaldehyde and drying in hot air (Legge 1934; see also Wolff and Heimann 1951). For the disinfection of hides Robertson (1932) recommended the use of H_2S ; anthrax spores are destroyed in 7-16 days. It is more usual, however, in Great Britain to treat them with 1/5000 $HgCl_2$ and 1 per cent. formic acid for 24 hours, or with 2 per cent HCl and 10 per cent. $NaCl$ for 48 hours, and then store them for 14 days. Hair and bristle should be autoclaved or exposed to warm formaldehyde vapour. (For information on other methods and disinfectants, see Hailer and Heicken 1948, 1950.)

Vaccination.—In 1879, Chauveau (1880*b*) found that an animal which survived inoculation with anthrax was more resistant to subsequent inoculations. The following year, Toussaint (1880*a, b*) successfully vaccinated sheep with defibrinated anthrax blood that had been heated for 10 minutes at 55° C. In 1881, Pasteur (1881*b, c*) introduced a method of vaccination, founded on the same principle as that which had been successful in diminishing the virulence of the organism of chicken cholera. He found that if the anthrax bacillus was cultivated in broth at 42-43° C., not only did it lose its power of forming spores, but it gradually decreased in virulence, till after 2 to 3 months it was no longer able to give rise to disease, even in the most susceptible animals. Pasteur prepared two vaccines; the first or *premier vaccin* was a subculture in broth from a strain that had been kept at 42-43° C. for 15 to 20 days; its virulence was such that it could kill mice and young guinea-pigs, but was unable to kill adult guinea-pigs or rabbits. The second or *deuxième vaccin* was a subculture after 10 to 12 days; it was much more virulent, being able to kill mice, adult guinea-pigs and a certain proportion of injected rabbits.

Arrangements were made with the President of the Agricultural Society of Melun for a trial of this new method under field conditions. Accordingly on May 5, 1881, on a farm at Pouilly-le-Fort, 24 sheep, 1 goat and 6 cows received their first vaccination, and on May 17 they were vaccinated a second time with the more virulent but still attenuated strain. On May 31, Pasteur and his co-workers, Roux and Chamberland, inoculated each of the vaccinated animals with a virulent culture of anthrax; at the same time a series of control animals consisting of 24 sheep, 1 goat and 4 cows were similarly inoculated. Two days later, the vaccinated sheep and the goat were in perfect condition; the control sheep and the goat were all dead. The 4 unvaccinated cows were suffering from severe cedema and fever; the 6 vaccinated cows had neither fever nor cedema. The following day one of the vaccinated sheep died, but at necropsy it was found to be carrying a foetus that had been dead for about a fortnight.

The success of this experiment led to the vaccination on a large scale of cattle and sheep in Europe, in South America, and in other countries. In 1894 Chamberland collected the statistics that were available for the years 1882 to 1893 inclusive (Table 156). It will be seen that before vaccination, the mean mortality of sheep was 10 per cent., of cattle 5 per cent. Chamberland calculated that vaccination had effected a saving of 5 million francs on sheep, and of 2 million francs on cattle.

mm in diameter. Later the centers may become slightly raised. Colonies on blood agar show hemolysis.

Physiology. The tetanus bacillus develops in plain or dextrose broth and in brain, meat, agar and gelatin media from which the air has been expelled by heating and excluded by some form of seal. If the depth of media is adequate, say 10 to 12 cm, no special seal is required, especially for the more viscous media. Growth occurs between 14° and 43° C, the optimum temperature is 37° C.

The growth of *Cl. tetani* is influenced greatly by the presence of associated microorganisms. In sugar-free media it may be grown in mixed cultures upon the surface of culture media in contact with air through the absorption of oxygen by the associated aerobes. But in dextrose broth the growth of the tetanus bacillus in mixed culture is likely to be inhibited by acid formation due to the associated bacteria. Sugar-free media, such as meat infusion peptone broth or deep meat or brain media, are therefore preferable for initial culture from contaminated material. Pure cultures may be isolated from these initial cultures by deep agar or surface culture methods.³ It has been supposed that an initial heating of the contaminated material simplifies isolation of the tetanus bacillus by destroying the vegetative cells of other microorganisms that may be present, this method may, however, leave the spores of the tetanus bacillus still badly mixed with those of other aerobic and anaerobic bacteria, some of which may be more resistant than the tetanus spores.

In pure culture, dextrose stimulates growth as in the case of the other anaerobes, although dextrose and other carbohydrates are not fermented by *Cl. tetani*. Sporulation is not inhibited by carbohydrates, however, as with the fermentative anaerobes, on the contrary, sporulation of the tetanus bacillus is accelerated in dextrose broth.

Growth in gelatin stab is slow at 22° C., but in ten days or so the characteristic inverted fir-tree type of growth is apparent. Incubated at 37° C. for two to three days, gelatin cultures usually fail to stiffen in the refrigerator. Coagulated proteins, such as blood serum and egg white, are very slowly liquefied. Deep brain and meat media may be slightly softened, but never digested fully, after several weeks a slight darkening occurs near the surface exposed to air. The tetanus bacillus is, therefore, only weakly proteolytic. Amino acids are, however, utilized readily but not by paired oxidation-reduction, glutamic acid, aspartic acid and serine, for example, are attacked directly with the formation of CO₂, NH₃ and acetic and butyric acids.⁴ Both amino acids and carbon compounds are readily dehydrogenated.⁵ In litmus milk there is reduction of the indicator and sometimes a slight precipitation of the casein. Nitrates are not reduced to nitrites, but hydrogen sulfide and indol are produced.

The tetanus bacillus has been grown in synthetic media and its growth requirements are relatively complex, including the amino acids arginine, histidine, tyrosine, valine, leucine, isoleucine and tryptophane, the bacterial

³ Cf. Reed and Orr. Proc. Soc. Exp. Biol. Med., 1941, 48:535, War Med., 1941, 1:493.

⁴ Clifton. Jour. Bact., 1942, 44:179.

⁵ Pickett. Jour. Biol. Chem., 1943, 151:203.

Later Sterne (1939) in South Africa introduced a vaccine prepared by suspending avirulent uncapsulated sporing bacilli in 50 per cent. glycerol saline. Tests on $2\frac{1}{2}$ million cattle and several thousand horses and sheep are reported to have shown that this vaccine gives as good protection as the saponin vaccine, and has the advantage of being safer and producing less reaction. In Great Britain a combination of the two methods is used, Sterne's strain being suspended in saponin.

Reference may be made briefly to Cienkowski's method, which consists of the use of two vaccines of spore-bearing bacilli attenuated by heat and standardized in virulence by repeated passage through gophers (*Zieselmaus*). It has been used chiefly in Russia, and also in Japan (Poppe 1922).

SOBERNHAIM'S COMBINED METHOD.—This is a method of combined active and passive immunization. Sobernheim (1902, 1904, 1906) prepared an immune serum by injection of cattle or sheep with cultures of increasing virulence. For his vaccine, he used a culture slightly attenuated by growth at 42.5°C . In practice, simultaneous injections are made, beneath the skin, of 10–16 ml. of immune serum and 0.5–1.0 ml. of vaccine. This method has been extensively adopted in Germany and South America, where it appears to have given good results. It has the merit of requiring injection on only one occasion instead of two; of conferring a passive immunity which protects the animal while an active immunity is developing; and of being attended by very little danger. It can be employed after an epidemic has broken out, without fear of rendering the animals more susceptible to infection.

None of the methods in use confers an immunity for more than 9 months or a year; hence vaccination must be repeated annually. It is extremely difficult to gather from the imperfect figures that have been reported what the value of vaccination really is. Instead of vaccinating alternate animals, and comparing the mortality of the vaccinated with the control animals in the same herd, the experiments have nearly all been made by vaccination of the entire herd, and comparing the mortality of the vaccinated animals with that of the non-vaccinated animals during previous years. The evidence is therefore circumstantial, and cannot be considered satisfactory.

On the whole, however, vaccination does seem to be of value in districts in which the disease is rife. It should never be employed in countries where merely sporadic cases occur, for the living bacilli that are injected may actually help to spread rather than to restrict the disease. Of the various methods referred to, the saponin Carbozoo vaccine seems to enjoy the greatest favour at present for the routine immunization of large animals.

No satisfactory vaccine is yet available for man, though there seems to be no reason why a vaccine prepared by Gladstone's (1948) technique should not be tried. Its use would, of course, be limited to those specially exposed to the risk of infection.

Serum Treatment.—Several workers have prepared an immune serum by injection of animals with cultures of increasing virulence, and have found it capable of conferring passive immunity on rabbits. Sobernheim, in particular, obtained satisfactory results both for protective and for therapeutic purposes with immune sheep and cattle serum, but speaking generally the serum treatment of animals has never been employed on a large scale.

In man, on the other hand, serum treatment has been extensively used. Slavo (1895) first immunized sheep, but later (1896, 1898, 1901) he found that the ass gave the most satisfactory results. In 1903 Slavo collected 164 cases of anthrax

experimental data have been presented in support of the theory of the axis-cylinder pathway,¹⁶ and as yet no conclusion may be drawn.

Tetanus toxin possesses a strong affinity for the cells of the central nervous system, as evidenced by the now classic experiments of Wassermann and Takaki.¹⁷ A mixture of toxin and brain substance can be injected into an animal without producing any toxic effect, the toxin apparently entering into a firm combination with some ingredient of the nerve substance. Not only the central nerve cells, but to some extent other tissue cells, are able to bind tetanus toxin. Subcutaneous inoculation is less likely to result fatally than direct inoculation into nerve tissues because some of the toxin is bound and prevented from reaching the highly sensitive nerve cells.

Pathogenicity. Tetanus is essentially an intoxication. The bacilli set up a localized infection and the toxin formed there is disseminated through the body and gives rise to the symptom-complex characteristic of the disease. Bacillemia may occur very rarely, however, and has been produced experimentally. The bacilli generally gain entrance to the tissues by means of a deep, dirty wound which may be relatively small, so small sometimes as to escape serious attention. The widespread occurrence of the tetanus bacillus would seem out of harmony with the relative infrequency of tetanus infection but, it may be noted, mere introduction of the bacillus into the body is not sufficient to produce the disease; the microorganisms must find favorable conditions for proliferation at the site of penetration. Experimentally, pure cultures of vegetative cells or spores that have been freed from toxin cannot germinate in uninjured tissues, but simultaneous inoculation with common saprophytes, such as *Bacterium prodigiosum*, or with irritant chemicals, such as calcium salts or lactic acid, enables the bacilli to grow and form toxin. As indicated elsewhere (p. 74), a sufficiently low oxidation-reduction potential is necessary for the germination of tetanus spores, and it is not unlikely that the potential of normal tissues is too high to allow germination but is reduced by injury.

Tetanus of the newborn or tetanus neonatorum is a consequence of infection of the umbilicus through septic midwifery. It is especially common among the Negroes of the southern states and in other races living under unhygienic conditions.

The tonic spasms characteristic of tetanus usually begin at the site of infection, and the initial symptoms may include headache and stiffness of the neck. The spasms may remain localized in mild infections, but usually they are general and involve the whole somatic muscular system. Postmortem findings are insignificant; other than a moderate congestion, the organs show no pathological changes and the initial lesion may, of course, be inapparent or small.

The incubation period of tetanus is variable and may range from two to fifty days. The case fatality is inversely related to the incubation time; it may be as high as 70 to 80 per cent or as low as 15 to 20 per cent. Death, if it occurs, follows relatively soon after the appearance of symptoms; the dictum of

¹⁶ Cf. Friedemann, Hollander and Tarlov: Jour. Immunol., 1941, 40:325, Roefe: Science, 1947, 105:180.

¹⁷ Wassermann and Takaki: Ber. klin. Wehnschr., 1898, 35:5.

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13,124 animals after operations or accidental wounds, without the occurrence of a single case of tetanus. During the same time two veterinary surgeons alone saw 139 cases of tetanus among animals which did not receive the treatment. The figures of Nocard and Labat added to Vaillard's data cover the cases of 16,917 animals receiving prophylactic injections; among them only one horse had tetanus. In this case the antitoxin was given five days after the wound and the attack was mild.

It is probable that in many cases tetanus is averted in man by the prophylactic use of antitoxin. Even though the disease is not prevented the incubation period is delayed and the disease may be very mild or remain localized. Precise statistical evidence involving comparable series of controls is not, of course, available, but the experience of the first World War was that with increased use of tetanus antitoxin there was not only a lower incidence of the disease but an increase in mild and chronic cases. Passive immunization provides antibody in the circulating blood which combines with toxin and renders it harmless. The avidity of the nervous tissue for tetanus toxin is very great, however, and symptoms may appear in spite of the presence of circulating antibody. Passive immunity is transient, of course, and repeated injections of antitoxin at six- to seven-day intervals must be made as long as danger of infection remains.

The Therapeutic Use of Antitoxin. Tetanus antitoxin appears to have but limited therapeutic value. It is obvious, of course, that symptoms result from damage to the nerve tissue and the administration of antitoxin will neither repair such damage nor displace the more avid nerve tissue already in combination with toxin. Reports on the therapeutic value of antitoxin are conflicting; some workers contend that intrathecal administration either alone or combined with intramuscular injection has a beneficial effect. The records of Cook County Hospital in Chicago, however, show that the therapeutic use of antitoxin has not reduced the mortality from tetanus there.²²

Active Immunization. In recent years some emphasis has been placed upon active immunization against tetanus, particularly by the French workers. In a summary of the results of twelve years' experience with active immunization of horses and men with formol toxoid, Ramon²³ states that in one cavalry unit in which tetanus was endemic more than 50,000 horses were immunized over a ten-year period and tetanus has practically disappeared, and in a million and a half human beings immunized with toxoid no case of tetanus has occurred.

With the beginning of the second World War, active immunization against tetanus was adopted by the armed services of France, Britain and the United States. Both fluid and alum-precipitated toxoid are used, in this country the former by the Army and the latter by the Navy, and appear to be equally effective though three doses of fluid toxoid are required as against two doses of alum-precipitated toxoid. No international standard exists for tetanus toxoid; the United States Army specified that the toxin contain at least 10,000 guinea pig MLD's per ml. and be detoxified with 0.4 per cent formalin; the final preparation must be atoxic for guinea pigs in 5 ml. amounts, and pigs receiving 1 ml. as an immunizing dose must be able to withstand 10 MLD of toxin at the

²² Calvin: Jour. Amer. Med. Assn., 1930, 94:1977.

²³ Ramon. Rev. Immunol., 1939, 5:477.

an entrance to the bacilli—tetanus neonatorum; and in mothers tetanus occurs, though rarely, as the result of infection in the post-partum genital tract. Infections of the face or head give rise to a peculiar form—cephalic tetanus—characterized by facial paralysis and dysphagia. Visceral tetanus is an uncommon form, in which infection appears to originate from the bacilli in the intestinal tract

In this country the disease is comparatively uncommon. From 1915 to 1924 the mean annual number of civilian deaths in England and Wales was 157.7, of which 123.2 were in men and 34.5 were in women. In the years 1931 to 1948, the number in men declined from over 90 to less than 50 a year—an incidence change from about 5 to 2.5 per million; in women the annual number varied between 18 and 29. Rural populations are more prone than urban (Conybeare and Logan 1951). In 1909 there were 1,373 deaths from tetanus amongst 732,528 deaths from all causes in 18 states of North America; of these 30.7 per cent. were in children under 1 year (Osler 1920). At one time the 4th July celebrations were followed by a large number of cases, usually resulting from blank cartridge wounds (Smith 1908). In other countries, particularly in the tropics where hygiene is primitive, the disease is more widespread.

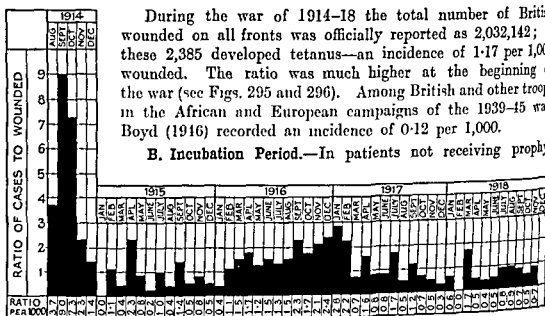


FIG. 295.

The ratio per 1,000 of cases of tetanus to wounded. (After Bruce.)

laetic injection of antitoxin the incubation period, after a severe wound, varies from about 2 to 30 days; the commonest time of onset is 7–10 days. During the 1914–18 war the incubation period lengthened—partly on account of the use of antitoxin prophylactically; thus in cases treated in home hospitals, the incubation period increased from an average of 11.8 days in 1914 to 50 days in 1918–19 (Bruce 1920). In the actively immunized, on the other hand, the incubation period is shortened (Boyd 1946; see p. 1975).

C. Fatality.—This depends on the nature of the wound, the site of infection, and the treatment accorded. Severe wounds, especially if deep, lacerated, or badly soiled, are characterized by a high mortality. Wounds of the upper extremities are more dangerous than those of the trunk or lower extremities (Bruce

end of six weeks.²⁴ More rapid methods of assay using mice have been suggested. For immunization three doses of 1 ml. each are given at intervals of three weeks and a stimulating dose one year later. The value of more than two doses is illustrated by Evans²⁵ who found that the mean antitoxin titer after two inoculations was 0.35 IU (International Units) but with a third inoculation ten months later the mean titer rose to 10 IU and eighteen months later was still 0.37 IU.

In practice this immunization appears to be highly effective. In the period 1942-45 only 12 cases of tetanus occurred in the United States Army, of which 6 were in unimmunized persons,²⁶ and 4 cases in the United States Navy of which 3 were in unimmunized persons; in contrast a tetanus rate of about 10 per 100,000 wounded prevailed in the Japanese army and navy which did not practice routine immunization. British experience in the Middle East war zone similarly indicated an effective immune response, though in most cases only two doses were given.²⁷ The success of active immunization, coupled with the low incidence of untoward reactions (reported to be 1 in 10,000 immunizations with improved toxoid), has suggested its more general application in civil life. In France, where active immunization to tetanus has been of more and earlier interest than elsewhere, it has been made compulsory as with diphtheria immunization.²⁸ In this country considerable interest has attached to combined tetanus and diphtheria immunization of children

GASEOUS GANGRENE²⁹

Gaseous gangrene is a syndrome often following dirty, lacerated wounds, especially those involving fractures. It is a characteristic complication of war wounds, and present knowledge of this affection was largely developed during the first World War. But this disease is by no means so rare in civil life as was

OUTSTANDING PATHOLOGICAL CHANGES IN GUINEA PIGS INOCULATED WITH ANAEROBES OR THEIR TOXINS

(Hall)

	Edema	Emphysema	Congestion	Histolysis
<i>Cl. tetani</i>	-	-	+	-
<i>Cl. septicum</i>	+	++	+++	+
<i>Cl. welchii</i>	++	+++	+	+
<i>Cl. novyi</i>	+++	-	-	-
<i>Cl. sordellii</i>	+++	-	+	-
<i>Cl. histolyticum</i>	-	-	++	+++
<i>Cl. chauvoei</i>	+	++	+++	+
<i>Cl. botulinum</i>	-	-	+	-

²⁴ Long Amer. Jour. Pub. Health., 1943, 33:53

²⁵ Evans Lancet, 1943, ii 316.

²⁶ Long and Sartwell Bull. U. S. Army Med. Dept., 1947, 7:371.

²⁷ Boyd and MacLennan Lancet, 1942, i 745.

²⁸ Bull. Office Internat. d'Hyg. Publique, 1940, 32:748

²⁹ See the general review by Danielson Trans. New York Acad. Sci., Ser. II, 1947, 9:297.

ANIMAL FÆCES.—Toledo (Toledo and Veillon 1891) demonstrated the presence of *Cl. tetani* in horse and in cow dung. Noble (1915) found it in 18 per cent. of 61 samples of horse fæces examined, but not in any of 21 samples of cow fæces. Sanfelice (1893) demonstrated it in 7 out of 23 samples of normal guinea-pig fæces. Fildes (1925a) isolated it from 34 out of 200 samples of normal horse fæces from London stables. Kerrin (1929) found it in 8 out of 53 samples of horse fæces, 4 out of 21 samples of cow, 6 out of 23 samples of sheep, 17 out of 37 samples of dog, 52 out of 141 samples of rat, and 6 out of 34 samples of hen fæces. Approximately half of the strains proved to be atoxic.

HUMAN FÆCES.—Pizzini (1898) demonstrated the presence of *Cl. tetani* in human fæces; he found it in 3 out of 10 samples from ostlers, and in 2 out of 90 samples from peasants. On the basis of these results, it has been generally assumed that contact with horses strongly predisposes to the carrier condition in man, but we doubt if this assumption is justified. In this country Tulloch (1919-20) found the tetanus bacillus in 5 out of 31 specimens of fæces, and Fildes (1925a) only twice in 200 specimens, while Kerrin (1928, 1929) failed to find it once in an examination of 304 samples of adult stools. Similarly Scheunemann (1931) in Germany was unable to isolate it from 50 samples of human fæces. Bandmann (1953) recorded its absence from 92 stools, and summarized some earlier investigations, in which the faecal carrier rates lay between 0 and 40 per cent. The tetanus bacillus must be ingested frequently with raw food, but the evidence suggests that it is an organism of passage, which does not find a natural habitat in the human intestine.

Reproduction of the Disease in Animals.

Tetanus can be reproduced by the inoculation of pure cultures or of toxin into mice, rats, guinea-pigs, rabbits, goats, sheep, horses, and monkeys. Cats and dogs are more resistant; birds and cold-blooded animals are highly resistant. The most susceptible animal, calculated on the amount of toxin per gram of body weight necessary to prove fatal on injection, is the horse. This is about 12 times as susceptible as the mouse; the guinea-pig is 6 times, and the monkey 4 times, as susceptible as the mouse (von Lingelsheim 1912, Sherrington 1917). On the other hand, the rabbit is twice, the dog 50 times, the cat 600, and the hen 30,000 times as resistant as the mouse (Kitasato 1891, von Lingelsheim 1912). For a description of the lesions produced in laboratory animals reference should be made to Chapter 36.

Mode of Infection in Tetanus.

Vaillard and Rouget in 1892 found that, when tetanus cultures were heated to 65-67° C for half an hour to destroy the vegetative bacilli and the toxin, the toxin-free spores remaining could be injected in large numbers into a guinea-pig without giving rise to the disease. The spores did not germinate in the tissues, but were rapidly taken up by the phagocytes, so that in 2 or 3 days they were completely ingested. When, however, the spores were protected from the phagocytes by being wrapped in filter paper, they germinated and gave rise to fatal tetanus. The same results could be obtained by inflicting a trauma at the site of injection sufficient to cause tissue necrosis or effusion of blood, or by creating a simple fracture. In the damaged tissue the spores were able to germinate, whereas in clean, aseptic wounds they were unable to do so. The presence of other organisms, particularly aerobic, in the wound also permitted the germination of the spores. From these observations it appears that toxin-free spores are innocuous, but that they can be activated by injuring the tissues or by a secondary infection.

These observations were extended by several workers. Thus Francis (1924) found that toxin-free spores were rendered pathogenic for guinea-pigs by the simultaneous

formerly thought. The increased number of injuries in automobile accidents is responsible for many cases of gangrene. Men injured about railroad tracks, either employees or vagrants, seem particularly prone to develop gaseous gangrene if not properly and promptly treated. Certain forms of peritonitis, appendicitis, intestinal obstruction, puerperal sepsis and postoperative infections (particularly after laparotomy) are etiologically closely related to it.

In the fulminating form of gaseous gangrene the muscles become filled with gas and with a serosanguineous exudate depending for its character upon the associated microorganisms, for this disease is nearly always a mixed infection of aerobes and anaerobes of several species. The accompanying table shows the chief pathological changes in guinea pigs inoculated with various anaerobes or their toxins.

THE FREQUENCY OF OCCURRENCE OF ANAEROBIC BACILLUS SPECIES IN GASEOUS GANGRENE

Species	Mixed Anaerobic Flora		Pure Anaerobic Flora	
	Cases	Per Cent	Cases	Per Cent
<i>Cl. perfringens (welchii)</i>	91	72	37	74
<i>Cl. sporogenes</i>	34	27	2	4
<i>Cl. edematiens (noveyi)</i>	33	26	6	12
<i>Cl. fallax</i>	26	21	3	6
<i>Cl. septicum</i>	12	9.5	1	2
<i>Cl. tetani</i>	11	8.7	—	—
<i>Cl. histolyticum</i>	8	6.3	—	—
<i>Cl. aerofaecium</i>	5	3.9	1	2
<i>Cl. putrificum</i>	2	1.6	—	—
<i>Cl. bifermentens (sordellii)</i>	2	1.6	—	—
<i>Cl. tertium</i>	1	0.8	—	—
Bacillus II of Ghon and Sachs	1	0.8	—	—

Weinberg and Séguin³⁰ studied 126 cases of gangrenous wound infection other than tetanus. Of these, 35 were phlegmonous and 6 of them yielded only aerobic bacteria; the remainder contained anaerobic bacilli. Thus aerobes are apparently able to produce gaseous phlegmons, but the number of such cases is small; gaseous infections are predominantly anaerobic and the role of aerobes is, as a rule, only accessory. Of the 12 cases of anaerobic infection, 90 contained both aerobes and anaerobes; 70 contained more than 1 anaerobe and 50 but a single anaerobic species. The frequency with which the various anaerobic species were found is summarized in the accompanying table. Similar data collected during the World War II by MacLennan³¹ showed somewhat different proportions but the same general trend, a predominance of *Cl. welchii*, *Cl. novyi* and *Cl. septicum*.

Clearly, then, the bacteriology of gaseous gangrene is complicated and

³⁰ Weinberg and Séguin: *La Gangrène Gazeuse*. Masson et Cie., Paris. 1918.

³¹ MacLennan: *Lancet*, 1943, i, 63, 94, 123, *ibid.*, 1944, ii 203.

areas of necrosis were produced, either by earth or by calcium chloride. Fildes considered that in the normal tissues the O-R potential is sufficiently high to prevent the spores from germinating; but that in areas of necrosis the potential falls to a point sufficiently low to permit of their germination.

Further observations by Fildes (1929*a, b*) and his colleagues (Knight and Fildes 1930, Campbell and Fildes 1931) greatly strengthened this view. Tetanus spores were unable to germinate in a medium of pH 7.0-7.6 unless the oxidation reduction potential was reduced to Eh + 0.1 volt or below. The Eh of the subcutaneous tissue of the guinea pig was found to be above this level. If, however, the partial pressure of oxygen in the tissues was lowered by keeping the animals in an atmosphere containing only 7 per cent., instead of the normal 21 per cent., of oxygen, then the Eh was reduced to a level at which germination of the spores in the presence of a mild activating agent such as aleuronat was often rendered possible. (See also p. 1384.)

From these observations we may form a conception of the genesis of tetanus in the body. When tetanus spores are introduced into a wound by contamination with earth, horse faeces, or other material, their fate depends largely on the presence or absence of certain accessory factors. Many of these have been described— notably trauma, hæmorrhage, tissue necrosis; chemicals such as lactic acid, saponin, trimethylamine, colloidal silicic acid, and ionizable calcium salts; and biological substances such as the toxins of *Cl. septicum* and *Cl. welchii*. Which of these factors is the more important it is impossible to say; recent work tends to ascribe less importance to mechanical injury, and more to calcium salts and the toxins of other anaerobes. The presence of a suitable accessory factor enables the spores to germinate and multiply in the tissues; it seems probable that this action is dependent on the production by tissue debilitants of an area of necrosis with a sufficiently low oxidation-reduction potential to permit the spores to germinate. A similar local debilitating action by gelatin, vaccine lymph, anti-toxic sera and bacterial vaccines is probably the reason for the tetanus that Smith (1908) records as following the injection of these substances. In post-operative tetanus the spores may be derived either from infected catgut (see Smith 1908, Mackie 1928, Mackie *et al.* 1929, Bulloch 1929, Savolainen 1950), imperfectly sterilized instruments or dressings, plasters and talcum powders, or some other source such as the intestinal or the respiratory tract (see Carbone and Perrero 1895, Motzfeldt 1912, Wright 1930, Tremewan 1946, Murray and Denton 1949).

Absorption and Mode of Action of Tetanus Toxin.

The absorption and mode of action of tetanus toxin has provided a problem of extraordinary interest to experimental workers. The field covered has been so wide that it is impossible to do more than give here an outline of some of the more important experiments.

When a guinea-pig dies of tetanus after local injection of a culture of *Cl. tetani*, the site of inoculation is rich in toxin (several thousand mouse m.l.d.; Francis 1924); but the blood is only slightly toxic, and the internal organs, even the central nervous system, where toxin might be expected to accumulate, are non-toxic (Rosenbach 1886, Kitasato 1889, 1891, Meyer and Ransom 1903). Intoxication does not appear to spread by the blood stream, because to produce fatal tetanus, more toxin is required by the intravenous than by the subcutaneous route; and with a given dose of toxin, the incubation period is longer after the intravenous injection.

Bruschettini (1890, 1892) induced tetanus in rabbits by the injection of toxin beneath the skin or into the sciatic nerve; suspensions of the organs were made and injected into

while, as stated by Weinberg and Séguin, there is in effect a typical form of gaseous gangrene, (a) it is not always produced by the same microorganism, (b) it is frequently caused by several associated agents, (c) it is often the complex result of the combined action of these principal anaerobic bacilli with various other bacteria which play an indeterminate accessory role.

The more important of the anaerobic bacilli associated with gaseous gangrene are discussed in the following sections.

1. THE VIBRION SEPTIQUE, CLOSTRIDIUM SEPTICUM

In 1877 and 1881 Pasteur, while studying anthrax, produced a septicemia in rabbits and guinea pigs by the inoculation of putrid blood from a cow. The affection could be communicated from individual to individual, and a sporulating, motile, rod-shaped anaerobe, considered by him "one of the vibrios of putrefaction" (the actively motile bacilli sometimes appear to be



Fig. 129. *Clostridium septicum* from pure culture. The tendency to form elongated vegetative cells is apparent Fuchsin, $\times 1050$.

curved), was regarded as the cause of the septicemia and named "vibron septicum."

In 1881 Koch described the pathological effects of a microorganism which he declared identical with the vibron septicum of Pasteur. But this bacterium failed to produce a septicemia in guinea pigs, and since its pathogenic effects were limited largely to the site of inoculation, Koch designated it "the bacillus of malignant edema."

Neither Pasteur's nor Koch's description would suffice now to identify with certainty the microorganisms in question. Fortunately, the original strain of Pasteur's "vibron septicum" has been maintained in France, so that its outstanding characteristics are well known. The lack of any such legacy from Koch in Germany, due to his failure to recover cultures, has led to an all but interminable discussion as to the properties of the "true bacillus of malignant edema." The vibron septicum is now generally known as *Cl. septicum*.

Morphology. The vibron septicum is a gram positive, sporulating, spindle-shaped rod, or filament, and in young cultures motile, with many

the opposite limb and finally the muscles over the whole body become affected. A small amount is probably absorbed by the lymph and carried to the blood stream. From this it is taken up by the motor nerve endings in different parts of the body. Hence the tetanus developing after intravenous injection of toxin is slower in its development, but is generalized from the first.

This conception of the genesis of tetanus was very severely criticized by Abel (1934) and his colleagues (Abel and Hampil 1935, Abel *et al.* 1935*a, b*, Abel, Evans and Hampil 1936, Firor and Jonas 1938, Abel *et al.* 1938, Abel, Firor and Chalian 1938).

Briefly, these workers objected to the hypothesis of axis cylinder carriage on the ground that (1) there is no evidence to show that the axis cylinders ever contain tetanus toxin; (2) there is no known mechanism that will explain how the toxin is transported in the axis cylinders, often for long distances, to the central nervous system; (3) the injection of a sub-lethal dose of toxin directly into the sciatic nerve of the dog does not give rise to tetanus, *provided measures are taken to prevent the escape of the toxin into the surrounding tissues*; and (4) the injection of antitoxin into the sciatic nerve, *provided none leaks into the surrounding tissues*, does not prevent the development of local tetanus after injection of toxin into the muscles of the leg.

A second hypothesis, which has been held by some workers, that the toxin is carried to the central nervous system by the endoneural and perineural lymphatic vessels, seems to be ruled out on anatomical grounds. There is no evidence to show that the neural lymphatics drain into the cerebrospinal fluid; on the contrary, they appear to drain directly into lymph nodes, which in their turn drain into the thoracic duct.

Discounting these two hypotheses, as well as that of the transference of the toxin in the tissue spaces of the nerve trunks by molecular forces, Abel adopted the view that the toxin reaches the central nervous system by the arterial circulation. Contrary to the findings of certain previous workers, he recorded that, when doses greater than the minimal lethal dose are used, a very high proportion of the toxin injected into the limb of a sheep may be demonstrated in the lymph-glandular and vascular systems. Minimal lethal doses of toxin are fixed by neural and other tissues, so that the toxicity of any tissue for experimental animals into which it is injected results entirely from the presence of blood or lymph containing free toxin. Secondly, Abel believed that tetanus toxin has a dual action on the central and on the peripheral nervous system. By means of multiple injections at various points of a limb, he produced local tetanus in dogs with amounts of toxin so small that generalized tetanus never developed. Intravenous toxin, on the other hand, produced both the local muscular rigidity and the reflex excitability of the muscles that characterizes generalized tetanus; whereas the application of toxin directly to the cord in the region of the anterior horn after a latent period of one to two hours produced the reflex excitability alone. The abolition of local tetanus by section of the appropriate nerve depends, not on the destruction of the path by which the toxin reaches the anterior horn cells, but on the abnormally low tonicity of the motor end-organs.

Harvey (1939) also recorded evidence in favour of a local action of toxin, depending on the occurrence of tetanus in locally intoxicated muscle after recent section of the motor nerve; and of after-discharge at the myoneural junction in tetanized muscle following electrical stimulation of the cut motor nerve (see also Schaefer 1944). These observations have not been confirmed (see Perdrup 1946). Harvey suggested that toxin acted by increasing the normal leak, and therefore the synthesis, of acetyl choline in the end-organs, and by decreasing cholinesterase production. Ambache, Morgan and Wright (1948*a, b*) describe what they consider a direct local effect of the toxin on the rabbit's eye, a non spastic paralysis of the cholinergic oculomotor nerve-endings. There is a concomitant decrease of acetyl choline in the iris and aqueous humour, but no change in cholinesterase.

peritrichous flagella. The ends are slightly rounded and the spores, which are oval, are usually median and swell the vegetative cell into a clostridium previous to their release. Spores are formed only in media not containing fermentable carbohydrate in excess. The long chains and filaments of these microorganisms which occur on the visceral surfaces of infected guinea pigs are of high differential value. Capsules have never been observed. Deep colonies in 1 per cent agar are transparent or semitransparent. Hemolysis occurs on blood agar.

Physiology. *Cl. septicum* is a strict anaerobe and develops readily in deep brain or tissue media, producing gas rather abundantly. These media are not discolored even in the presence of metallic iron. Gelatin is liquefied, but coagulated serum and other proteins are not digested or blackened. Hydrogen sulfide is produced but indol is not. Dextrose, levulose, galactose, maltose, lactose and salicin are fermented; media not containing one of these sugars support only slight growth. Sucrose, inulin, mannitol and dulcitol are not fermented. The fermentation of salicin and non-fermentation of sucrose allow the biochemical differentiation of *Cl. septicum* and *Cl. chauvei*, for the latter does not ferment salicin but does ferment sucrose.

Antigenic Structure and Toxin. Strains of *Cl. septicum* are immunologically related but distinct. Four groups have been distinguished on the basis of the somatic antigen, and subdivisions may be made by the flagellar antigen. These bacilli are immunologically related to *Cl. chauvei*. The toxin formed, however, is specific, but a relatively weak lethal agent. The MLD for mice is about 0.005 ml. Bernheimer³² has prepared a dialyzable medium containing casein hydrolysate, cystine, tryptophane, glutamine, biotin, thiamine, nicotinic acid, pyridoxine, glucose, thioglycollic acid and inorganic salts which supports the growth of some strains with the formation of 400 to 500 mouse LD₅₀ doses of toxin per ml. When injected into animals it produces a gelatinous edema and some local necrosis of the tissues. According to Kellaway, Reid and Trethewie³³ the toxin has a specific cardiac action in the cat and rabbit, producing a fall in systemic and a rise in venous blood pressure, in the cat a specific constriction in the pulmonary and coronary circulations, with edema of the lungs and loss of fluid from the circulation.

Pathogenicity. *Cl. septicum* does not occur in gaseous gangrene of man as frequently as some of the other anaerobic bacilli but has been found in such affections both alone and in mixed cultures. It has been recovered from gaseous infections in cattle and may be one of several microorganisms responsible for blackleg, usually considered a specific disease due to *Cl. chauvei*. It has also been found in gaseous infections of hogs and other domestic animals. Experimentally the vibron septique is strikingly pathogenic for chickens, pigeons, rabbits, guinea pigs, rats and mice. In such animals the bacteria develop rapidly, producing gas and a reddish, serous edema. They invade the adjacent tissues and the circulation, producing a septicemia which is usually fatal within twenty-four to forty-eight hours; sublethal doses do not produce any reaction. Impression smears from the tissues, and especially from the liver.

³² Bernheimer. Jour. Exp. Med., 1944, 80:321.

³³ Kellaway, Reid and Trethewie: Australian Jour. Exp. Biol. Med. Sci., 1941, 19:297.

radioactive materials. In this connection we may note that Barnes and Trueta (1941), who immobilized the limbs of rabbits in plaster, found that several lethal doses of toxin could be injected into such limbs without ill effect. When after some days the plaster was removed tetanus followed. If we assume an intact blood circulation, it appears that toxin was not absorbed into the blood capillaries; the restoration of movement in the leg might have promoted absorption by the lymphatics, or intraneural ascent as a result of muscular movements.

Summarizing, we may say that we are still ignorant of the exact mechanism by which tetanus toxin is absorbed, and of the way in which it exerts its action on the body cells. The main hypothesis is that the toxin is absorbed by the motor nerve endings, and carried up the nerve trunks to the anterior horn cells of the central nervous system, which are then stimulated to produce muscular contractions: there is independent evidence of centripetal transmission of substances in nerve trunks, taking place presumably in the spaces between the nerve fibres. The weight of evidence is against the view that the toxin acts locally on the motor nerve endings, and centrally by reaching the anterior horn cells via the lymphatic vessels and the blood stream.

Immunity to Tetanus.

Natural.—Mammals vary in their susceptibility to tetanus; some, such as the mouse, the monkey, and the horse are highly susceptible; others, such as the dog and the cat, are much less susceptible. Most birds and cold-blooded animals are extremely resistant. Examination of the blood of susceptible and partially susceptible animals shows that, though the majority of cattle contain more than 1/500 unit of antitoxin per ml., no antitoxin is present in the blood of man, horses, dogs, pigs, monkeys, or rodents. Sheep and goats may contain small quantities (Coleman 1931, Coleman and Meyer 1926, Ramon and Lemétayer 1934, 1935). The frequent presence of antitoxin in the blood of ruminants may be causally associated with the comparative resistance of these animals to tetanus. It is suggested that in ruminants tetanus bacilli multiply and form toxin in the digestive reservoirs which precede the true stomach, and that the toxin so formed, partly modified perhaps by the products of bacterial fermentation, is absorbed and gives rise to the production of antitoxin. Examination of the blood of naturally resistant animals has proved it to be devoid of antitoxin (Vaillard 1892); hence their immunity cannot be attributed to the presence of this antibody.

Marie (1897) injected a rabbit intravenously with 1,000,000 mouse M.L.D. of toxin. Blood was removed at frequent intervals, defibrinated, and injected in 1 ml. quantities into mice. For the first 17 hours the blood proved toxic, but after this time it became innocuous; the toxin had disappeared therefore from the circulation in less than a day. Vaillard (1892), on the other hand, who injected a hen with 20 ml. of toxin, found that it persisted in the blood stream for several days. In the susceptible rabbit the toxin is supposed to be absorbed from the blood by the motor nerve endings, and carried to the cord, where it gives rise to disease. In the non-susceptible hen the toxin appears to be absorbed either not at all, or only slowly; hence tetanus fails to develop.

An interesting contribution to the study of natural immunity was made by Wassermann and Takaki (Wassermann 1898, Wassermann and Takaki 1899), who found that the brain, and to a less extent the spinal cord, of normal animals was able to neutralize tetanus toxin. A mixture of toxin and brain suspension proved harmless on injection into animals. The amount that could be neutralized de-

usually show elongated filaments or chains as contrasted with the single bacilli found in animals killed with *Cl. chauvei*.

Antitoxic sera which are prophylactic and, to some degree, curative may be prepared by injection of *Cl. septicum* toxin into horses. The antisera do not have the high antitoxin content that is found in antitetanic sera. Polyvalent commercial sera for prophylactic and therapeutic use in wound infections often contain antibodies to *Cl. septicum*.

2 CLOSTRIDIUM WELCHII (CLOSTRIDIUM PERFRINGENS)

Clostridium welchii was first cultivated by Achalmé in 1891 and supposed by him to be the cause of articular rheumatism. In 1892 Welch and Nuttall isolated this bacillus from the foamy organs of a cadaver and called it *Bacillus aerogenes capsulatus*. Found by Frankel the following year, it was designated

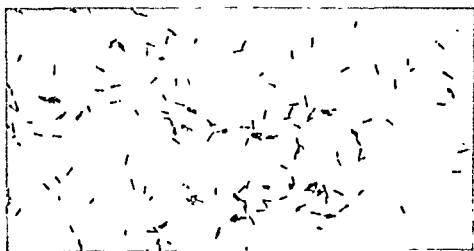


Fig. 130. *Clostridium welchii* from pure culture. Note the relatively smaller size of these bacteria and the central spores. Fuchsin; $\times 1050$.

Bacillus phlegmonis emphysematosae, and in 1897 Veillon and Zuber called it *Bacillus perfringens*. Sometimes called Frankel's bacillus in Germany, *Cl. perfringens* in France and *Cl. welchii* in English-speaking countries, it is designated *Cl. perfringens* by Bergey.

Morphology. *Cl. welchii* is a plump, non-motile, gram-positive rod of variable length, occurring in chains and singly. Capsules are usually present in preparations made from the organs or body fluids. Spores are formed sparingly and only in the absence of fermentable carbohydrates, they are centrally located, rarely subterminal, and do not swell the vegetative cell in which they are formed. Isolated colonies in deep agar are compact, opaque, white or grayish white biconvex disks. On blood agar the round, smooth, opaque, entire-edged colonies are relatively large, 2 to 5 mm. in diameter, and surrounded by a zone of hemolysis.

Welch's bacillus is a strict anaerobe and grows readily in deep brain, meat infusion broth, agar and gelatin media. Its growth in sugar-free media is

nerves. When the antitoxin is not given till after the formation of toxin has begun, it will be too late to prevent the absorption by the nerves.

It follows that there are rigid conditions governing the usefulness of antitoxin. The most important of these is that it must be allowed to come into intimate contact with the toxin. Unless it does so it is valueless. It is known that the nervous tissue of mammals has a strong affinity for tetanus toxin; it has no such affinity for tetanus antitoxin. Once the toxin has gained entrance to the nerves it can no longer be neutralized, unless the antitoxin is itself injected into the nerves. Meyer and Ransom (1903) injected antitoxin in large quantities intravenously into a cat, and at the same time injected a dose of toxin into the sciatic nerve; the cat developed tetanus. The nerve tissue acts as a barrier separating the toxin from the antitoxin; and nothing short of enormous doses of antitoxin in the blood stream can save the life of an animal if once the toxin has gained access to the nerves. We have, in fact, the anomaly of an animal, whose blood contains sufficient antitoxin to save the life of hundreds of mice, itself dying of tetanus. If the antitoxin is injected directly into the central nervous system, there is some evidence that it is able to neutralize the toxin and prevent further spread of the disease (Roux and Borrel 1898). In more carefully controlled experiments Friedemann, Hollander and Tarlov (1911) demonstrated the superiority of intraventricular over intravenous antitoxin in preventing local tetanus. Roux and Borrel also showed that an actively immunized rabbit, capable of withstanding large doses of toxin subcutaneously or intravenously, yet succumbs as readily to the intracerebral injection of toxin as a normal rabbit.

It should be noted that Abel (Abel *et al.* 1938, Abel and Chalian 1938) distinguished two stages in the action of toxin—adsorption to the nerve cells, and fixation. Fixation coincided with the appearance of tetanic symptoms (See also Pelloja 1950). In the sheep, antitoxin given intravenously after demonstrable absorption of lethal amounts of toxin by the nervous tissue, but before symptoms of descending tetanus had appeared, had a curative action.

There is little evidence (see Lahiri 1939) of immunity naturally acquired in the absence of an attack of the disease similar to the antitoxic immunity acquired as the result of subclinical infections with *C. diphtheriae* (Chapter 61); although the serum of man, horse, cattle and sheep is reported to contain antitetanolyisin, and that of cattle and sheep to contain antitoxin (Lemétayer and Nicol 1945).

Diagnosis.

The bacteriological diagnosis of tetanus is often difficult, especially in civilian patients, where the site of infection may be small.

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Cultural Methods are of more importance. The pus or wound scrapings are seeded into cooked meat medium, or into heart or liver digest broth containing a small portion of sterile rabbit kidney, or other material designed to initiate a high reducing intensity in the medium. After 5 days' anaerobic incubation at 37° C, if the typical bacilli are visible microscopically, the culture is heated to 70–75° C. for 20 minutes to destroy non-sporing bacilli, and plated out on liver or serum

greatly restricted. Optimum conditions for growth are provided by media containing fermentable carbohydrates, but such cultures are often short-lived because of the lack of spore formation and the destructive action of the formed acids on the vegetative cells. Brain and meat media are not blackened normally, but the presence of metallic iron produces a distinct discoloration. Gelatin is liquefied but coagulated serum or egg is not digested. Hydrogen sulfide is produced, indol is usually said to be negative but its formation is uncertain.

Nutritive requirements are complex, and semisynthetic media which support growth include casein hydrolysate or 19 amino acids together with pantothenic acid, thiamine, nicotinic acid, riboflavin, biotin, folic acid, pyridoxine, adenine, guanine, uracil, inorganic salts including those of manganese and iron, together with glucose.³⁴ The production of a toxin (see below) requires two additional substances, one found in enzymatic digests of certain proteins, and the other an alcohol-soluble constituent of pancreas; glyceryl phosphocholine appears to be in part responsible for the activity of the latter.³⁵

Acid and gas are produced in glucose, maltose, lactose and sucrose, neither mannitol nor salicin is fermented; some strains ferment inulin and some glycerol. Broth cultures containing fermentable sugars become markedly turbid with abundant gas formation, and many cultures, possibly all at certain stages, become stringy and viscid. Milk is fermented with a characteristic "stormy" evolution of gas, followed by coagulation of the casein due to acid formation, and the curd is shortly torn to shreds by the continued evolution of gas within. The curd is not digested. This "typical" reaction is considerably modified by incomplete anaerobiosis, gas production may be slow and the solid curd is torn only slightly if at all. Under optimum conditions 3.8 times the volume of the milk may be evolved as gas; hydrogen predominates during the early stages of the fermentation and carbon dioxide in the later stages.

Types. Although *Cl. welchii* strains from gaseous gangrene form the same toxin, they are not immunologically homogeneous. Henderson³⁶ has observed that the Wilsdon types (see below) are homogeneous with respect to heat-stable antigen with the exception of Type D in which he found six kinds of antigens in thirteen strains. Rodwell³⁷ has shown that a number of subtypes of each of the Wilsdon types may be distinguished by agglutination and precipitin tests and there are some cross reactions between types. The common antigen appears to be a capsular polysaccharide; this substance has been prepared by Svec and McCoy.³⁸ As yet, however, serological identification of *Cl. welchii* is not practical. Four biochemical types have been suggested on the basis of differences in the fermentation of glycerol and inulin, but these fermentative differences do not appear to be correlated with other variable characteristics.

Toxigenic bacilli closely resembling *Cl. welchii* and having immunologically related toxins have been isolated from lower animals. These are the lamb dysentery bacillus (*Bacillus agni*), a bacillus causing a disease of sheep called

³⁴ Boyd, Logan and Tytell: Jour. Biol. Chem., 1947, 167:879.

³⁵ Adams, Hendee and Pappenheimer: Jour. Exp. Med., 1947, 85:701.

³⁶ Henderson: Jour. Hyg., 1940, 40:501.

³⁷ Rodwell. Australian Vet. Jour., 1941, 17:58

³⁸ Svec and McCoy. Jour. Bact., 1944, 48:31.

nerves. When the antitoxin is not given till after the formation of toxin has begun, it will be too late to prevent the absorption by the nerves.

It follows that there are rigid conditions governing the usefulness of antitoxin. The most important of these is that it must be allowed to come into intimate contact with the toxin. Unless it does so it is valueless. It is known that the nervous tissue of mammals has a strong affinity for tetanus toxin; it has no such affinity for tetanus antitoxin. Once the toxin has gained entrance to the nerves it can no longer be neutralized, unless the antitoxin is itself injected into the nerves. Meyer and Ransom (1903) injected antitoxin in large quantities intravenously into a cat, and at the same time injected a dose of toxin into the sciatic nerve; the cat developed tetanus. The nerve tissue acts as a barrier separating the toxin from the antitoxin; and nothing short of enormous doses of antitoxin in the blood stream can save the life of an animal if once the toxin has gained access to the nerves. We have, in fact, the anomaly of an animal, whose blood contains sufficient antitoxin to save the life of hundreds of mice, itself dying of tetanus. If the antitoxin is injected directly into the central nervous system, there is some evidence that it is able to neutralize the toxin and prevent further spread of the disease (Roux and Borrel 1898). In more carefully controlled experiments Friedemann, Hollander and Tarlov (1941) demonstrated the superiority of intraventricular over intravenous antitoxin in preventing local tetanus. Roux and Borrel also showed that an actively immunized rabbit, capable of withstanding large doses of toxin subcutaneously or intravenously, yet succumbs as readily to the intracerebral injection of toxin as a normal rabbit.

It should be noted that Abel (Abel *et al.* 1938, Abel and Chalian 1933) distinguished two stages in the action of toxin—adsorption to the nerve cells, and fixation. Fixation coincided with the appearance of tetanic symptoms. (See also Pelloja 1950) In the sheep, antitoxin given intravenously after demonstrable absorption of lethal amounts of toxin by the nervous tissue, but before symptoms of descending tetanus had appeared, had a curative action.

There is little evidence (see Lahn 1939) of immunity naturally acquired in the absence of an attack of the disease similar to the antitoxic immunity acquired as the result of subclinical infections with *C. diphtheriae* (Chapter 61); although the serum of man, horse, cattle and sheep is reported to contain antitetanolyisin, and that of cattle and sheep to contain antitoxin (Lemétayer and Nicol 1945).

Diagnosis.

The bacteriological diagnosis of tetanus is often difficult, especially in civilian patients, where the site of infection may be small.

Microscopical Examination should be made where possible of the pus in the wound, and special note taken of bacilli with round, terminal spores. The presence of "drumstick" bacilli is *not*, however, pathognomonic of infection with *Cl. tetani* (see, *e.g.*, Boyd and MacLennan 1912). The organisms may be present in such small numbers that they may be altogether overlooked.

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"struck" (*Bacillus paludis*), and a bacillus responsible for an enterotoxemia of sheep (*Bacillus ovispastus*). Wilsdon³⁹ has proposed that these be designated *Cl. welchii* but that four types be distinguished *Cl. welchii* Type A—the gaseous-gangrene bacillus, *Cl. welchii* Type B—the lamb-dysentery bacillus, *Cl. welchii* Type C—the "struck" bacillus, and *Cl. welchii* Type D—the bacillus of enterotoxemia of sheep. Unless otherwise indicated, however, the name *Cl. welchii* always refers to the gas-gangrene bacillus, or Type A.

Toxins. It has been shown that, as a group, these bacilli form seven immunologically distinct toxins and that the observed interrelationships of the toxins formed by the various types are attributable to the sharing of one or more of these components⁴⁰ The effects produced by these toxins are as follows:

The α toxin is hemolytic, lethal to mice on intravenous injection, and produces necrosis on intradermal injection into guinea pigs and rabbits. Termed by Wuldon the W factor and designated by some workers as the χ toxin.

The β toxin is not hemolytic, mice injected intravenously develop spasmodic twittings and die almost immediately, and it produces skin necrosis in guinea pigs and rabbits. Termed by Wilsdon the Z factor

The γ toxin is not hemolytic, does not produce skin necrosis in guinea pigs, and is lethal to mice.

The δ toxin is hemolytic but is not lethal to mice and does not produce skin necrosis.

The ϵ toxin is nonhemolytic, but produces skin necrosis in guinea pigs and rabbits and is lethal to mice. Termed by Wilsdon the X factor

The θ toxin is hemolytic and lethal and probably produces necrosis in high concentrations. It is oxygen labile and thermolabile and very similar in properties and immunological specificity, though not identical with, streptolysin O. It is the same as Prigge's toxin.

The η toxin has lethal activity only. It has been found in only one strain (Leech) of Type A as yet examined and its occurrence or absence in the other types is not definitely established.

The optimal conditions of pH, incubation time and composition of the medium differ from one toxin to another, and a type producing more than one toxin will produce only those for which the conditions are optimal. The distribution among the Wilsdon types of the ability to form these toxins is indicated in the accompanying table. The terminology of the toxins has been somewhat confused owing to differences in the terms used by various workers, that used here has been generally agreed upon.

Of these the α toxin has been of greatest interest since it is associated with the virulence of the bacilli. It was observed independently by Nagler⁴¹ and by Seiffert⁴² that an opalescence is produced in human serum by the addition of filtrate containing the α toxin. This is known as the *Nagler reaction*. On the

of fat. It was then shown by Macfarlane and Knight¹² that an enzymatic split-

³⁹ Wilsdon Inst. Animal Path., Cambridge, 2nd report of the Director, 1931, p. 53.

⁴⁰ See the review by Oakley *Bull. Hyg.*, 1943, 18 781.¹¹ Nagler. Brit. Jour. Exp. Path., 1939, 20: 473.⁴² Seiffert *Ztschr. f. Immunitätsf.* 1939, 96 515.¹³ Macfarlane, Oakley and Anderson. *Jour. Path. Bact.*, 1941, 52: 99.¹⁴ Macfarlane and Knight *Biochem Jour.*, 1941, 35 882.

Vaillard (1892) confirmed the value of iodized toxin, and succeeded in immunizing rabbits so that the animals were able to withstand the intravenous injection of pure toxin; but he was unable to satisfy himself that antitoxin was curative (Vaillard 1891). A mouse receiving 0.5 ml. of this rabbit antitoxin was protected for a few days, but the immunity had gone in about a fortnight; antitoxin given a few hours after tetanus was manifest had no effect (Vaillard 1891; see also Tizzoni and Cattani 1891).

Antitoxin was later produced on a large scale by the injection of horses with toxin-antitoxin mixtures (Buxton and Glenny 1921). The toxin was over-neutralized, and a series of injections was given at intervals of a few days over a period of some months. Detoxification with formol (Descombey 1924) is now widely used. The action of the

formol is to deprive the toxin of its toxicity, while having little effect on its antigenic value. After a series of initial injections with this formolized product—sometimes referred to as anatoxin or formol toxoid—crude toxin itself can be injected. The actual technique of preparing the toxoid need not detain us here. Usually about 0.4 per cent. of formol is added to the toxin, and the mixture is incubated at 37°–39° C. till it is sufficiently detoxified. About 20 ml. of formolized toxin should prove non-toxic to the guinea-pig on subcutaneous inoculation (Hosoya *et al.* 1931, Wilcox 1934). Some workers precipitate the toxoid with alum or zinc chloride, and some add tapioca or 0.5 per cent. CaCl_2 in an endeavour to increase its antigenic efficiency (see Glenny *et al.* 1926, Ramon *et al.* 1931, Ramon and Lemétayer 1932, Bergey 1934).

The standardization of tetanus antitoxin has presented considerable difficulties, due in part to the liability of tetanus toxin to deterioration. This difficulty was overcome by the establishment of stable standard antitoxins, to which were assigned arbitrary unit values of antitoxic potency. In 1928, the Permanent Commission on Standardization of the League of Nations chose the U.S. National Institutes of Health (N.I.H.) standard (Rosenau and Anderson 1908) as the international standard preparation. The French and the German national standards were also considered. The three national units were such that 1 German unit = 66 American = 3,750 French units (Prausnitz 1929). Although the U.S. dried preparation of antitoxin was adopted, unfortunately the international unit was made, not equal to the established N.I.H. unit, but to

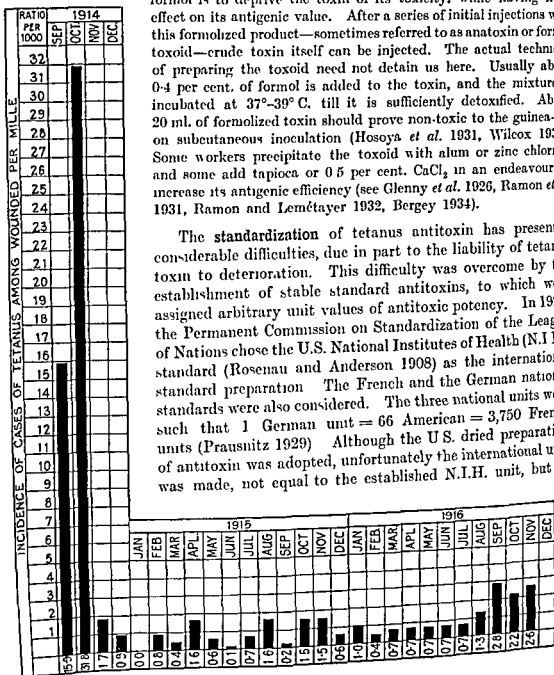


FIG. 296.

The incidence of tetanus per 1,000 wounded in Home Hospitals during 1914-16, showing the fall in incidence after Oct., 1914, when the use of antitetanic serum became general. (After Bruce.)

ting of free lecithin into . . . the toxin appears to be a . . . has been suggested that the α toxin may be estimated by measurement of the liberation of soluble phosphorus under standard conditions.

In addition to the α toxin and θ toxin noted above, *Cl. welchii* forms a collagenase which has been designated the κ toxin which breaks down muscle by dissolution of its collagen and reticulin structure⁴⁵; the part it plays in the pathology of the infection is not clear. It does not appear to be as important as the α toxin, however, for while antiserum to the α toxin alone is protective, anticollagenase alone is not.⁴⁶

TOXINS FORMED BY THE WILSDON TYPES OF *CL. WELCHII*

Type	Toxins						
	α	β	γ	δ	ϵ	θ	η
<i>Cl. welchii</i> Type A (bacillus of gas gangrene) . . .	+	—	—	—	—	+	+
<i>Cl. welchii</i> Type B (<i>Bacillus agni</i>)	+	+	+	±	—	+?	?
<i>Cl. welchii</i> Type C (<i>Bacillus paludis</i>)	+	+	+	+	—	+?	?
<i>Cl. welchii</i> Type D (<i>Bacillus orelaxans</i>)	+	—	—	—	+	+?	?

In general the toxins formed by *Cl. welchii* appear to account in very large part for the observed histopathology. Robb-Smith,⁴⁷ for example, has compared that in naturally occurring infection in man, in experimentally infected animals and in normal human muscle exposed to the action of filtrates *in vitro* and found the histopathological changes substantially the same in all three.

There appears to be no simple method of typing *Cl. welchii* and the presence or absence of the various toxins must be demonstrated. The differentiation of the toxins suggested by Wilsdon³⁹ is relatively simple but too crude for most purposes. A precise method of determining the toxic components of a filtrate using monospecific antisera is outlined by Oakley.⁴⁰

Pathogenicity for Man. *Cl. welchii* is, perhaps, the most important cause of gaseous gangrene and is found either alone or mixed with other anaerobes in the majority of cases of this disease. Tissue injury is a usual, perhaps essential, preliminary to infection, but once the bacilli are established they invade the surrounding tissue rapidly. They apparently travel along the interstitial tissue of the muscle and are often found beyond the gangrenous area. The large amount of hyaluronidase produced would seem to be related

⁴⁵ Oakley, Warrack and van Heyningen. *Jour. Path. Bact.*, 1946, 58:229.

⁴⁶ Evans. *Brit. Jour. Exp. Path.*, 1947, 28:24.

⁴⁷ Robb-Smith. *Lancet*, 1945, ii:362.

as soon as possible after the receipt of the wound. The type that calls for this treatment is the dirty lacerated wound, contaminated with street mud, earth, or animal faeces, especially if accompanied by severe bruising of the tissues or fracture of the bones. It is advisable to inject a portion of the serum in two or three places around the actual lesion. The wound itself should preferably be excised: if this is impossible it should be cleansed and thoroughly drained. If the wound is severe and is likely to take some time to heal, it is well to repeat the injection every week. Should symptoms of tetanus develop, large doses—30,000 units or more—must be given every day, partly around the wound, partly intravenously, and, if desired, intrathecally.

From animal experiments there is not the least doubt of the protective value of antitoxin, but in man the evidence is necessarily indirect. This may be summarized as follows:

(1) The prophylactic injection of antitoxin within a few hours of the receipt of a wound appears to diminish greatly the chances of tetanus. The incidence during the war of 1914–18 is recorded in Figs. 295 and 296. The sudden fall in November, 1914, can in all probability be ascribed to the prophylactic use of antitetanic serum that was introduced about the middle of the preceding month. The rise towards the end of 1916 may have been fictitious; Bruce ascribes it to improved diagnosis, particularly of local tetanus, which was largely overlooked at the beginning of the war. The subsequent fall in 1917 and 1918 may perhaps be related to the more effective prophylactic serum treatment which was commenced in June, 1917, but more probably to the practice of early excision of wounds which was introduced about the same time.

(2) When it does not prevent the development of tetanus it appears to lengthen its incubation period. The average incubation period in soldiers of the 1914–18 war treated in home hospitals rose from 11·8 days in 1914 to 48·0 days in 1917; and a direct comparison of the average incubation period in patients who had received, and those who had not received, a prophylactic injection of antitoxin after having been wounded led to the same conclusion; in the inoculated this period was 45·5 days, in the uninoculated 10·9 days. In this connection it must be remembered that the passive immunity conferred by a single injection of antitoxin wears off in about a fortnight or three weeks. During this period the patient is unlikely to develop tetanus. But once this is past, he is probably just as susceptible as a person who has never been inoculated at all. Hence if tetanus bacilli are still present in the wound, they may give rise to disease. If all patients had been given repeated doses of antitoxin at intervals of a week till their wounds healed, it is possible that these late cases of tetanus would have been greatly reduced in number.

(3) Tetanus developing in persons who have received a prophylactic injection appears to be less fatal than in uninoculated persons (Bruce 1920) (Table 159)

TABLE 159

SHOWING THE FATALITY OF TETANUS IN PATIENTS WITH AND WITHOUT ANTITOXIN PROPHYLAXIS.

	No of Cases.	Died.	Case Fatality per cent.
Prophylactic serum	890	203	22·6
No serum given . .	559	298	53·3

It is interesting to note that the average fatality amongst the inoculated during the war fell far more rapidly than amongst the uninoculated. This can probably be accounted

to this rapid spread, but a number of studies have indicated that there is little or no relation between the invasiveness of *Cl. welchii* and at least *in vitro* titers of hyaluronidase.

Although most commonly found in gangrene, *Cl. welchii* has also been observed in closed abscesses in uterine infections, and in infections of the gastro-intestinal, genito-urinary and biliary tracts. It has been isolated from the blood during life, but septicemia in man is much less common than in experimental animals, although blood invasion occurs frequently in man during the agonal period or immediately following death. Study of the "foamy organs" sometimes observed at autopsy has shown that the presence of gas in the internal organs shortly after death is often attributable to an invasion by this microorganism.

Cl. welchii is a normal inhabitant of the human intestine and is constantly present in small numbers; in fact, it has been used to a certain extent in Europe, as an indicator of fecal pollution of water (p 253). The toxemia of acute intestinal obstruction has been attributed by some to the proliferation of *Cl. welchii* in the bowel followed by absorption of formed toxin, but it is now clear that such a relationship does not exist. This bacterium is, however, found with some frequency in gangrenous appendicitis and it has been reported that antitoxin is of value in the treatment of perforative appendicitis.

Since infection with *Cl. welchii* is frequently characterized by gross blood destruction, jaundice and anemia, a possible relationship between this bacterium and various anemias has been of some interest. A pernicious and fatal anemia may be produced in experimental animals by intratibial inoculation of culture or a temporary but severe anemia by inoculation of filtrate. Both natural and experimental infections, therefore, lead to the development of a severe anemia which is probably due to the continuous release of the hemolytic toxin.

Pathogenicity for Animals. Natural infections in lower animals with *Cl. welchii* types have been referred to above. The occurrence of the gas-gangrene bacillus, however, is rare, local abscesses have been observed in dogs and rabbits following injury. Experimentally guinea pigs, pigeons and mice, less so !

killed a few minutes after intravenous incubated at 37° C., gas is produced in a few hours through the body and the phenomenon of "foamy liver" reproduced. This phenomenon is not strictly specific for *Cl. welchii*; it may be produced by similar inoculations with several other anaerobes, though the results are less striking. The pigeon is susceptible and is used for the standardization of toxin and antitoxin.

The classic *welchii* toxin, the α toxin, is not a powerful one; the MLD for a mouse is usually about 0.25 ml. of liquid culture. Antitoxins may be produced, however, which have both prophylactic and therapeutic value, and *welchii* is included in polyvalent antitoxic sera for gas gangrene. Antitoxin to the α toxin appears to be far more important than that to the θ toxin.⁴⁸ It may be noted that it is difficult to develop agglutinins for *Cl. welchii* and an antibacterial immunity does not protect against infection.

⁴⁸ Evans: Brit. Jour. Exp. Path., 1943, 24:81.

antitoxin used was large—2,000 units per kilo of monkey. This corresponds in man to 120,000 units administered in a single dose. To give this, using a potent preparation containing 3,000 units per ml., would necessitate the injection of 40 ml.—an amount in excess of that which could be administered intrathecally at one time. The average daily dose given in human practice—often by two or three routes—has been from 1,000 to 30,000 units; larger doses have not often been employed. If the quantities suggested by Sherrington's work are really necessary—and experience suggests that smaller doses are inadequate—then it would seem desirable to devote attention to the production of more potent antitoxic sera than those which we now possess.

Firor (1940) obtained similar results to those of Sherrington in dogs given 2 M.L.D. of toxin. Intrathecal antitoxin by the cisternal route saved 51 of 70 dogs (73 per cent.), and by the lumbar route 19 of 30 dogs (63 per cent.), whereas intravenous antitoxin saved only 5 of 20 dogs (25 per cent.).

The conclusion from this experimental work (see also Friedemann, Hollander and Tarlov 1941) finds some support in the experience of clinical observers who have succeeded in curing severe tetanus by the use of very large doses of anti-serum, sometimes administered by the cisterna magna (see Paterson 1930, O'Carroll 1931, Bell 1931, Nabarro 1932). Nabarro, for example, injected no fewer than 633,000 units of antitoxin within the space of 9 days, while Paterson used nearly 2 million units.

Even more suggestive, however, are the figures reported by Yodh (1932) in Bombay. He progressively increased the dosage of antitoxin for patients with tetanus, and the number of routes by which it was given, as indicated in Table 161, and observed a progressive decrease in case fatality. The cases were not strictly consecutive. Of a total of 229 cases treated by Yodh himself, 15 were not given serum treatment for one reason or another, sometimes because they were moribund. Since the distribution of these cases in point of time is not stated, it is impossible to know how much allowance to make on the figures for the serum-treated patients. The results, however, suggest strongly that the intrathecal administration of serum

TABLE 161

EFFECT OF ROUTE AND DOSE OF SERUM INJECTIONS IN HUMAN PATIENTS SUFFERING FROM TETANUS (Yodh 1932).

Mode of Serum Treatment.	No. of Patients	No died	Case fatality per cent.
20,000 units per diem by <i>im.</i> and <i>iv.</i> routes . .	49	38	77.6
30,000–40,000 units for 1st day, 20,000 units later, by <i>im.</i> and <i>iv.</i> routes	80	52	65.0
Larger doses of serum by <i>im.</i> , <i>iv.</i> , and lumbar intrathecal routes	22	14	63.6
Total of 40,000 to 400,000 units by <i>im.</i> , <i>iv.</i> , <i>sc.</i> , and cisterna magna routes	112	53	47.3

im. = intramuscular: *iv.* = intravenous: *sc.* = subcutaneous.

by the cisterna magna route is beneficial. Clinically, some improvement was generally noticeable within 24 hours of the cisternal injection.

From Sherrington's results on monkeys and Yodh's results on human beings, it would appear that intrathecal administration of serum is more beneficial than

3. CLOSTRIDIUM NOVYI (CLOSTRIDIUM OEDEMATIENS)

The third important anaerobe in gaseous gangrene was probably first discovered by Novy in 1894 in a study of "malignant edema" in guinea pigs, and was designated "*Bacillus oedematis malignia* Nr. II." It was named *Bacillus novyi* by Migula in 1900. In 1915 Weinberg and Séguin isolated several strains of this bacillus, but first regarded it as a new species and named it *Bacillus oedematiens*. The French name has been used by European workers although the bacillus is properly known as *Clostridium novyi*.

Cl. novyi is noteworthy not only for its importance in gaseous gangrene, but also because of its strong, soluble exotoxin, which compares in potency with

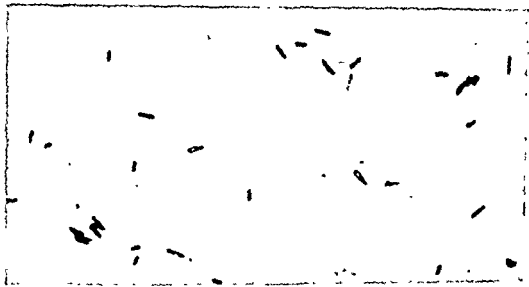


Fig. 131. *Clostridium novyi* from pure culture. The slight tendency to curvature is apparent in some of the vegetative cells. Note the subterminal spores and the absence of free spores in large numbers. Fuchsin; $\times 1050$.

the toxin of the diphtheria and tetanus bacilli, and for which an equally powerful antitoxin can be produced.

Morphology. Novy's bacillus is a large, relatively thick rod, 2.5 to 10 μ in length and 0.8 to 1 μ in breadth, and occurs singly and in chains. In cultures navicular and curved forms are found; in animal transudates the shorter form predominates. Its numerous spiral flagella, which often become tangled in "bouquets," have been emphasized in nearly all the published descriptions. The rod is non-motile under ordinary conditions of examination, for movement is markedly inhibited in the presence of air. Subterminal spores are produced but sparsely as a rule and best in nonfermentable media. The bacillus is gram-positive.

Young colonies in deep dextrose agar have a yellowish, opaque, irregular center surrounded by a delicate corona of short filaments. Later the colony clears, the center becomes cloudy, and is surrounded in forty-eight hours with a corona of tangled filaments. Surface colonies are extremely delicate, flattened, transparent, bluish gray, with irregular contours, and there is slight hemolysis on blood agar.

Physiology. *Cl. novyi* is a strict anaerobe, and grows well at 37° C. in

properly spaced intervals raises the antitoxin content of the blood within a few months to the level usually obtained by the injection of a prophylactic dose of antitoxin; and there is good reason to suppose that a given blood level indicates a far higher degree of immunity in the actively than in the passively immunized person. Thus d'Antona and Valensin (1937) found that actively immunized guinea-pigs with blood levels of 0.012 to 0.05 unit per ml. withstood 200 M.L.D. of toxin, while passively immunized animals with 0.2 to 0.5 unit per ml. either died or developed severe local tetanus (see also Sneath *et al.* 1937). The difference may in part be due to a better distribution of antitoxin in the actively immunized animal and to the rapid production of antitoxin in an animal conditioned by the previous antigenic stimulus. That the antibody-forming apparatus is conditioned in this manner is clear from the results of injecting toxoid some months after the primary series of injections. Not only is the antitoxin content raised to higher levels than the maxima reached after the previous injections, but the levels are maintained for longer periods. The response to this later stimulating dose is very striking; the antitoxin content of the blood rises rapidly, and titres of over 10 units per ml. are often reached—more than 100 times as high as those following the primary series of inoculations. There are great individual variations in response, both to the primary and secondary injections (see Ramon and Zoeller 1933, Sacquepée and Jude 1937, Boyd 1938, Marvell and Parish 1940, Prigge 1940, Evans 1941, 1943, Peshkin 1941, 1944, Fraser *et al.* 1943).

Reinforcing injections are also effective when given after longer periods. Thus Miller, Ryan and Beard (1949) recorded levels of 0.1 unit per ml. of serum in children given doses of alum-precipitated toxoid after 4–7 years. Both Regamey and Schlegel (1951) and Bigler (1951) tested primary immunity of 10 years' duration. Regamey and Schlegel's figures (Table 162) indicate a high degree of responsiveness to reinforcing doses after 10 years;

TABLE 162

SHOWING THE DURATION OF RESPONSIVENESS TO REINFORCING DOSES OF TETANUS
TOXOID INDUCED BY THREE PRIMARY INJECTIONS
(After Regamey and Schlegel 1951)

Years since Primary Inoculations	Number of Subjects Tested	Mean Number of Antitoxin Units per ml.			
		Days after Reinforcing Dose			
		0	4	6	8
1	26	0.70	1.27	9.9	18.9
2–3	10	0.52*	2.55	39.6	69.4
4–7	6	0.55	1.32	17.6	63.4
8–9	10	0.22	0.74	25.1	86.0
10	8	0.28	0.63	16.5	90.0

* Minimum individual titre in this group, 0.0075; in all other groups, minima were 0.035 or more.

and show that the speed of response to reinjection with fluid toxoid is best after 2–3 years. In a study of 300 children and infants, Bigler observed a progressive decline in response with lapse of time after primary immunization, but when three primary doses were given, even after 10 years the reinforcing dose induced protective concentrations of antitoxin within 7 days.

Effect of Simultaneous Immunization with other Antigens.—It is administratively convenient to mix toxoid with enteric vaccines, pertussis vaccines, or diphtheria toxoids. The response to toxoid or alum-precipitated toxoid is reported by Maclean and Holt (1940), Miller and Saito (1942), Greenberg and Fleming (1947, 1948), and Regamey and Schlegel (1951) to be enhanced by the presence of vaccine. Schiebel (1944), on the other hand, recorded a depression of antigenic potency of both diphtheria and tetanus toxoids (adsorbed to $Al(OH)_3$) when injected together into guinea-pigs, as compared with the potency of

ordinary media and especially abundantly in the presence of a fermentable sugar. Meat and brain are not darkened, the former may be turned slightly pink or bleached. Gelatin is liquefied, but coagulated serum and egg are not digested. McLeod and Gordon⁴⁹ have reported that *Cl. novyi* may be distinguished from the other sporulating anaerobes by culture on blood agar containing benzidine, the colonies are blackened due to the presence of hydrogen peroxide. Nitrates are not reduced and indol is not formed, but hydrogen sulfide is produced. Acid is produced very slowly in litmus milk, after ten to thirty days' incubation a fine flocculent clot appears which is not digested. Dextrose is fermented but lactose is not, the latter property serves to differentiate *Cl. novyi* from *Cl. septicum* and *Cl. chauvei*. There is not complete agreement as to the other fermentation reactions.

Antigenic Structure and Toxin. Three immunologic types of *Cl. novyi* have been defined by Scott, Turner and Vawter⁵⁰ and designated Type A, Type B and Type C.

The toxin formed by this organism is the most potent of the gaseous gangrene bacilli toxins, in contrast to *Cl. welchii* whose filtrates contain 4 to 5 mouse MLD per ml. the lethal dose of *Cl. novyi* filtrate for the mouse is about 0.005 ml. Lecithinase and hemolysin activity are present, and six components have been identified by Oakley, Warrack and Clarke.⁵¹ These are designated the α , β , γ , δ , ϵ and ζ toxins of which the α toxin is the classic lethal toxin. The distribution of these corresponds to the immunologic types noted above and is illustrated in the accompanying table from Oakley *et al*

DISTRIBUTION OF TOXINS AMONG *CL. NOVI* TYPES

Activity of Toxin	Designation	<i>Cl. novyi</i> Types		
		Type A	Type B	Type C
Lethal, necrotizing	α	+	+	-
Hemolytic, necrotizing, lecithinase	β	-	+	-
Hemolytic lecithinase	γ	+	-	+?
Oxygen-labile hemolysin	δ	+	-	-
Opalescence in lecithovitellin	ϵ	+	-	-
Hemolysin	ζ	-?	+	-

Pathogenicity for Man. The relatively frequent occurrence of *Cl. novyi* in gas gangrene has been noted earlier. The disease is characteristically a toxemia, although septicemia is not rare. Like *Cl. welchii*, Novy's bacillus is often a terminal invader. In pure infections there is less tissue destruction than with *Cl. welchii* or *Cl. septicum*. The postmortem findings consist mainly in a massive localized edema, with neither the extensive gas production of the former nor the sanguineous necrosis of the latter.

⁴⁹ McLeod and Gordon: Jour. Path. Bact., 1940, 50: 167.

⁵⁰ Scott, Turner and Vawter: Proc. 12th Int. Vet. Congr., 1934, 168.

⁵¹ Oakley, Warrack and Clarke: Jour. Gen. Microbiol., 1947, 1: 91.

Army, only one occurred among half a million wounded; in 6 of the 12, the soldier had not been immunized, and in 2, no reinforcing dose had been given. The rapid application of efficient surgery and of local and general chemotherapy no doubt contributed to the results in both allied armies, but the most important single factor appears to have been active immunization. The improvement on the results in 1914-18 cannot be attributed to fighting on sparsely infected terrain. Thus Glenn (1946) in Manila recorded that, among over 1100 wounded non-immunized civilians admitted to hospital for treatment in the same area as that occupied by the U.S. troops, 156 cases of tetanus occurred. Long (1948) cited an incidence of 1 per 100 wounded in the non-immunized Japanese army during 1940-44; though here again differences in the efficiency of non-specific prophylaxis must also be considered.

Clearly military personnel should be actively immunized. A case may also be made for special classes of civilian persons at risk, such as agricultural workers, persons with chronic ulcers of the lower limbs, habitually barefoot people in hygienically primitive communities, and pregnant women—the last as much to protect the infant against tetanus neonatorum by placentally transmitted maternal antitoxin as to protect the mother against puerperal tetanus. The case for routine immunization of children, at least in highly developed urban communities where the incidence of tetanus is low, is less clear and must be weighed against the disadvantage of imposing perhaps one more immunization on the child, or risking an ineffective immunizing stimulus by the use of multiple mixed antigens; and against the advantages and disadvantages of antitoxin prophylaxis.

As regards passive prophylaxis, antitoxin should certainly be given to non-immunized wounded; to actively immunized persons who are severely shocked or wounded; and when treatment has been delayed or when no reinforcing doses have been given in the previous 4-5 years. When combined passive and active prophylaxis or therapy has been carried out, the active immunity should be reinforced within a few months by another course of active immunization. A reinforcing dose only upon wounding appears to be sufficient specific prophylaxis in those with a good basic active immunity. Regamey and Schlegel (1950) believe that in these circumstances even a reinforcing dose is unnecessary, toxin from the infecting bacilli themselves providing the stimulus.

COMBINED ACTIVE AND PASSIVE PROPHYLAXIS

The administration of antitoxin and toxoid to ensure passive protection during the lag in the development of the secondary response has the disadvantage that the antitoxin may "blanket" the toxoid and thereby impair its antigenicity. Blanketing of this kind has been repeatedly demonstrated in the guinea-pig (Ramon and Zoeller 1933, Otten and Hennemann 1939, Regamey and Aegerter 1951, Wolters and Dehmel 1951). Otten and Hennemann found that the retardation of active immunity in their animals was best overcome when toxoid injections were made one day before the serum. In actively immunized rabbits, there was no suppression of the secondary response when the toxoid and antitoxin were injected into a separate site (Miller and Ryan 1950; see also Lemétayer *et al.* 1950). Combined prophylaxis is probably justified in the severely wounded if care is later taken to augment the active immunity by a fresh course of immunization (Ericsson 1948, Sachs 1952). (See also Chapter 50.)

We may note here a curative effect of toxoid in animals without basic immunity. Kreck (1949) records that toxoid, given 2 to 4 hours before injection of mice with living *Cl. tetani*, prolongs the survival time of the mice, and even prevents the appearance of tetanus in some of them. Lemétayer and his colleagues (1950) found that, though toxoid could not displace tetanus toxin from combination with nerve tissue either *in vivo* or

Pathogenicity for Animals. Natural infections due to *Cl. novyi* have been observed in guinea pigs, cattle, horses and hogs. Guinea pigs, rabbits, rats, mice, cats, sheep, horses and pigeons are susceptible to small doses of culture. Subcutaneous, intramuscular and intravenous inoculations reproduce the disease experimentally. Toxicity and pathogenicity are easily lost, however; Novy's original strain, which still survives in several laboratories, has long since failed to kill experimental animals. This is true also of strains isolated within five to ten years.

The action of the toxin freed from bacteria is very similar to that of whole cultures. Sublethal, subcutaneous doses of toxin or culture produce a peculiar non-hemorrhagic, gelatinous local edema which reaches its maximum in two or three days. It may be followed by small, superficial hemorrhages, after which it is slowly absorbed, leaving a slightly sclerotic scar. Such lesions appear not to form open phlegmons, as in the case of *Cl. welchii* cultures, and may also be contrasted with those of the vibron septique and *Cl. chauveii*, which, if they appear at all, are always fatal. Washed cultures are harmless.

Antitoxin has been produced in rabbits, sheep and horses by successively increased doses of toxic filtrates. The antitoxin has prophylactic and, to some extent, therapeutic value under experimental conditions, and is now represented in several polyvalent American sera or anaerobic infections.

4. CLOSTRIDIUM HISTOLYTICUM

Among the new species of bacteria discovered by Weinberg and Séguin in war wounds, none is of more interest than *Cl. histolyticum*, so named because

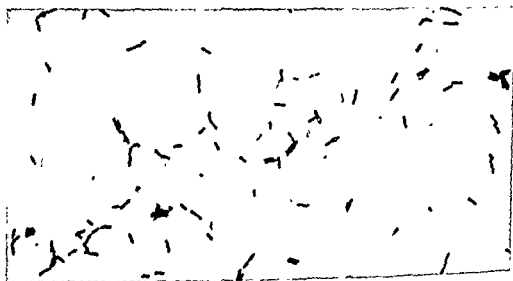


Fig. 132. *Clostridium histolyticum* from pure culture. Note the characteristic short rods with rounded ends and the clostridial subterminal spores. Fuchsin, $\times 1050$.

of its remarkable liquefying action upon living tissues. It may be somewhat more common in gaseous gangrene than indicated earlier. It has also been recovered from soil and human feces and from poisoned arrows.

Morphology. *Cl. histolyticum* is a gram-positive motile rod, 3 to 5 μ long and 0.5 to 0.7 μ wide, that forms subterminal clostridial spores. In smears from lesions it appears generally in the form of single or paired short rods

Prophylactic injection of antitoxin is recommended in operations on the horse. Nocard (see Mohler and Eichhorn 1911) reported that in 2,727 horses which were given serum after various operations not a single case of tetanus developed, whereas during the same period 259 cases developed in horses that were not inoculated (number not stated). A dose of not less than 1,000 units has proved successful. The passive immunity so conveyed lasts longer in the horse than in other animals because the serum is homologous, and is therefore excreted more slowly (Mohler and Eichhorn 1911). Buxton and Glenny (1921) immunized horses actively by 3 injections at intervals of 3 days of a toxin-antitoxin mixture. A month later the animals withstood 2,000 guinea-pig M.L.D. of crude toxin (see also Glenny, Hamp and Stevens 1932).

Tetanus toxoid is now used as an immunizing agent in horses which require protection. Two injections are given, at an interval of four to eight weeks. A reinforcing dose is advisable when animals are to be exposed to unusual risks. Oakley (personal communication) recommends the use of alum precipitated toxoid as a more consistently effective immunizing agent than formol toxoid.

Therapeutic injection of serum has not been followed by satisfactory results (Mohler and Eichhorn 1911).

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with rounded ends. The flagella, often more than 20 in number are peritrichal. Deep agar colonies vary, according to the consistency of the medium, from compact lobulate globules in 2 per cent agar to fluffy semi-transparent or even cottony balls in lower concentrations. Surface colonies are minute, round dew drops and are hemolytic on blood agar.

Physiology. Originally described as an obligate anaerobe *Cl. histolyticum* is capable of a delicate transparent growth upon the surface of meat infusion agar and is perhaps best regarded as microaerophilic or as a facultative anaerobe.

This bacillus is actively proteolytic, not only is gelatin liquefied but meat and brain and coagulated serum and egg are digested. In older cultures a precipitate of tyrosine crystals appears. Nitrates are not reduced and indol is not formed. No carbohydrates are known to be fermented, in spite of statements to the contrary regarding dextrose. The action on milk is slow, but after several days a soft clot is usually formed and then slowly digested. *Cl. histolyticum* is, clearly, a proteolytic type.

Pathogenicity. Infection with *Cl. histolyticum* alone is probably a rare occurrence, mixed cultures with other anaerobes and aerobes appear to be the rule both in war wounds and in infections observed in horses. Most pure cultures of this bacillus are pathogenic under experimental conditions for rabbits, guinea pigs, mice and rats, but there is considerable difference between strains. Subcutaneous inoculation of 1 or 2 ml. of a twenty-four-hour broth culture generally produces a local tumefaction followed in twenty-four to forty-eight hours by complete sloughing of the overlying skin, then, as a rule, healing slowly occurs. Intramuscular inoculation causes swelling, followed by progressive myolysis. If the gluteus muscle of a guinea pig is selected for the inoculation, it may be entirely denuded from the bone within twenty-four to forty-eight hours. The tissues literally drip away, and in some cases the limb may be disarticulated. Curiously there is often little or no intoxication of the animal, but death usually follows through peritonitis due to perforation of the peritoneum. There is occasionally invasion of the blood stream, but generally septicemia does not occur. There is never any gas formation in such pure infections.

Bacteria free filtrates have a lytic action which can be demonstrated if sufficiently large quantities (5 ml.) are injected. The most characteristic effect is the formation of a sterile hematoma filled with uncoagulated blood in which the red corpuscles are still intact. According to Pasternack and Bengtson¹² deep intramuscular inoculation produces an edema which disorganizes and separates the tissue, while gross lesions are observed only occasionally following intravenous inoculation. Agglutinating and antitoxic sera have been produced.

5 CLOSTRIDIUM SPOROGENES

Clostridium sporogenes, which in pure culture is a harmless saprophyte, is included here because it is frequently associated with the pathogenic anaerobes in mixed gangrenous infections, very possibly because it is so widely distributed in nature. It has frequently been confused with the pathogenic forms, not only have cultures labeled something else proved to be *sporogenes*, but there has

¹² Pasternack and Bengtson: Pub. Health Repts., 1940, 55:775.

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been a tendency to regard "atoxic variants" or "atoxic strains" of pathogens as *Cl. sporogenes*. On the other hand, the presence of "atoxic variants" of pathogenic species is in many cases attributable to mixed cultures containing *sporogenes*. The spores of this microorganism are unusually hardy and invariably survive with those of the pathogens or even after the pathogenic spore-formers are killed in preliminary selective heating.

Morphology. *Cl. sporogenes* is an actively motile, gram-positive, slender rod 3 to 7 μ in length and 0.6 to 0.8 μ in breadth, with rounded ends. The cells occur individually, in pairs, in short chains, and sometimes in filaments. The spores are oval, eccentric to subterminal, and swell the vegetative cell. The flagella are peritrichal. The deep agar colonies have the appearance of woolly balls with a dense, compact center. Surface colonies on blood agar are hemolytic, transparent and usually rhizoid, or ameboid, with a slightly raised center; they appear moist and at first may resemble minute dewdrops.

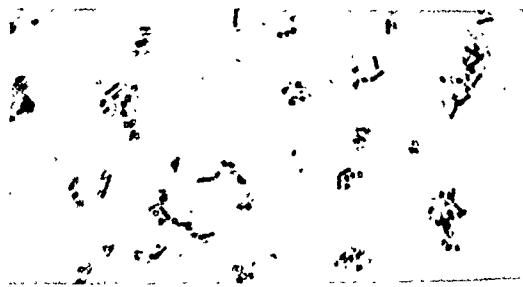


Fig. 133. *Clostridium sporogenes* from pure culture. Note the close morphological resemblance of this species to the pathogenic forms. Fuchsin; $\times 1050$.

Cl. sporogenes requires strictly anaerobic conditions for growth and will grow upon all the ordinary media. It has been cultivated in synthetic solutions containing tryptophane, leucine, tyrosine, arginine and phenylalanine, together with an unknown substance termed "sporogenes vitamin."⁵³ Its optimum temperature is 37° C. but it will grow at temperatures as high as 50° C.

This bacillus is actively proteolytic; it produces blackening and digestion of brain and meat media, coagulated egg and serum. An excess of fermentable sugar delays or inhibits this proteolysis and the presence of metallic iron or certain iron salts accelerates it. Tyrosine crystals are not obvious. Gelatin is liquefied and blackened, hydrogen sulfide is produced, but indol production is doubtful and nitrates are not reduced to nitrites. Reports on sugar fermentations are conflicting. According to Bergey (1939), acid and gas are formed from dextrose, levulose, galactose and maltose, while lactose, sucrose, salicin and inulin are not fermented. Growth on milk is at first slow, in forty-eight to

⁵³ Fildes and Richardson. Brit. Jour. Exp. Path., 1935, 16:326.

CHAPTER 78

GAS GANGRENE: CLOSTRIDIAL INFECTIONS OF MAN AND ANIMALS

GAS GANGRENE IN MAN

In the pre-antiseptic days the formation of sloughs, the occurrence of massive necrosis, and the onset of gangrene, so frequently followed upon any extensive operative procedure that they were regarded as more or less inevitable features of surgical practice. With the advent of antiseptic surgery, and the consequent healing of wounds by first intention, these processes became less and less common, till eventually even the names by which they were described took on an archaic air. It was therefore not unnatural that when gas gangrene appeared in the early days of the war of 1914-18, many surgeons were confronted with it for the first time. It was the older surgeons, who had had experience of pre-Listerian methods, that recognized its true nature, and that pointed out the impossibility of preventing it by the modern aseptic methods. Nevertheless, though the disease was old, its study had to be taken up anew. The problems were attacked with such success that by the end of the war gas gangrene had ceased to be of more than minor importance. In peace time the disease is uncommon, but is occasionally met with after severe compound fractures, and during the puerperium, particularly after illegal abortions (see Hill 1936).

In this chapter we can give only an outline of the original bacteriological investigation of gas gangrene, for a full description the reader is referred to the monograph of Weinberg and Séguin (1918), and to the Report of the Anaerobic Committee (see Report 1919).

Bacteriology.—In 1914 the incidence of gas gangrene amongst the British Expeditionary Force amounted to over 12 per cent. of the number wounded; of these 20-25 per cent. died. By 1918, owing to earlier evacuation of the wounded, excision of wounds, the employment of an antiseptic technique, and other measures, the incidence had fallen to under 1 per cent. (Report 1919). Indeed, in the Base Hospitals in 1918, amongst a total of about 25,000 wounded reported on by Bowlby (1919), only 84 patients developed serious or massive gas gangrene—an incidence of 0.34 per cent. In the French and German armies the incidence appears to have been rather higher. The incidence of gas gangrene in the 1939-45 war varied with the theatre of fighting. Among U.S. troops it ranged from 0 to 45 per 1,000 (Smith 1949), the higher figures usually being associated with land fighting, and, presumably, with contaminated soil (see Smith and Gardner 1949).

Examination of the wound of a man dying of gas gangrene reveals a number

seventy-two hours a clot is formed and progressive liquefaction occurs with abundant gas and acid formation until the serum is completely digested.

Pathogenicity. There is no authentic record of a natural infection attributable to *Cl. sporogenes* alone. It has been claimed that the bacillus is a factor in certain intestinal disorders, but its frequent occurrence in the intestinal tract of healthy men and animals is not in accord with this position.

In animal experiments relatively small doses are required to produce lesions. Less than 5 ml. of a young dextrose broth culture injected subcutaneously in guinea pigs, which are the most susceptible experimental animal, usually results in only a local manifestation. In a few hours the immediate overlying hair loosens, the skin becomes gangrenous and raised slightly over an area of subcutaneous tissue digestion in which a small amount of gas appears. Such animals usually show no systemic involvement and the lesion heals in a few days, leaving a necrotic scar which heals slowly. The reaction to intramuscular injection is only slightly more severe.

The most obviously important of the pathogenic manifestations of *Cl. sporogenes* (and probably of other putrefactive anaerobes) is that of a mutual acceleration in metabolism which occurs during growth with the more definitely pathogenic anaerobes, especially *Cl. welchii*, *Cl. septicum* and *Cl. novyi*. While the presence of various aerobes is in some degree stimulating to the growth of obligate anaerobes (due partly, as Pasteur suggested, to the absorption of oxygen, but also to other unknown factors) the presence of putrefactive anaerobes greatly enhances the pathogenicity of the non-putrefactive pathogens. The proteolytic forms supply protein split products which the fermentative types are unable to elaborate so rapidly. It may be noted that, while an admixture of a proteolytic culture reduces the minimum fatal dose of a non-proteolytic anaerobic experimentally, the admixture of *sporogenes* filtrate with toxic filtrates of *Cl. welchii* or *Cl. novyi* has no such action; there may, in fact, be a diminution of toxicity.

BLACKLEG (CLOSTRIDIUM CHAUVEI)

Blackleg, also known as quarter evil and symptomatic anthrax (not to be confused with anthrax, Chapter 27), is an important, widespread, acute disease affecting cattle. It occurs wherever cattle are kept and is prevalent throughout the United States with the possible exception of the Southern Atlantic and Eastern Gulf States. The name blackleg, like gaseous gangrene, has been applied to affections due to various anaerobes; in some instances *Cl. septicum* or, rarely, *Cl. novyi* is found, but the principal cause is *Cl. chauvei*. Just as *Cl. welchii* (Type A) is not involved in natural infections of lower animals, so *Cl. chauvei* has never been shown to be responsible for any human infection.

Although the bacilli had been earlier observed and the disease transmitted by the injection of the serous fluid from an infected animal into a healthy animal, *Cl. chauvei* was cultivated and its causal relation to blackleg established by Arloing, Cornevin and Thomas in 1887.

Morphology. *Cl. chauvei* is a gram positive, motile, sporulating rod. The size is variable, ranging from 3 to 8 μ , in length and about 1 μ in breadth. The cells occur singly as a rule, in contrast with *Cl. septicum*, there is little

characterized by foul, sero-purulent infection of the depths and crevices of the wound, without progressive involvement of muscle, the predominant organisms were proteolytic and non-toxicogenic clostridia, and *Cl. welchii*. In 146 cases of true gas gangrene, the pathogenic clostridia were as follows: *Cl. welchii* (57 per cent.), *Cl. oedematiens* (37.7 per cent.), *Cl. septicum* (20.0 per cent.), *Cl. tetani* (13.1 per cent.), *Cl. histolyticum* (6.9 per cent.), *Cl. bifermentans* (4.8 per cent.) and *Cl. fallax* (1.4 per cent.). As the fighting moved from desert to more cultivated country, the incidence of gas gangrene was doubled and the percentage of *Cl. welchii* infection increased. The frequency of the pathogenic clostridia when they were present singly in wounds was as follows: *Cl. welchii* 34.3 per cent., *Cl. oedematiens* 17.1 per cent., *Cl. septicum* 4.8 per cent., *Cl. histolyticum* and *Cl. bifermentans*, each 0.7 per cent. In other cases more than one species was isolated. Anaerobic streptococci (see Chapter 67) were found in 8.8 per cent. of cases. Other surveys in the 1939-45 war revealed substantially the same distribution of pathogenic clostridia (Zeissler 1944), excepting that Stock (1947) and Smith and George (1946) recorded an incidence of 4 per cent. or less for *Cl. septicum*. In India, Dhayagude and Purandare (1949) found no *Cl. oedematiens* in their patients with gas gangrene, and 20 and 36 per cent. of *Cl. histolyticum* and *Cl. septicum* respectively.

It should be noted that *Cl. botulinum* has been identified in wounds (Hall 1945), in some cases giving rise to clinical botulism (Davis *et al.* 1951, Thomas *et al.* 1951).

Summing up, we may say that gas gangrene may be caused by a number of different anaerobic bacteria, usually clostridia, but sometimes by anaerobic streptococci. Usually the clostridia are present either in combination with each other or with aerobic organisms. The three most important anaerobes are *Cl. welchii* (*B. perfringens*), *Cl. oedematiens*, and *Cl. septicum* (*Vibrio septique*); of less importance are *Cl. bifermentans*, *Cl. fallax*, *Cl. histolyticum*, and *Cl. sporogenes*. The presence of pathogenic clostridia in a wound, however, is not necessarily indicative of a gas gangrene infection. Most commonly they appear to be simple contaminants of wounded tissues; they are frequently associated with cellulitis, and only infrequently with true gas gangrene, i.e. clostridial myositis.

Reproduction of Gas Gangrene in Animals.

The experimental disease caused by *Cl. welchii*, *Cl. septicum*, and *Cl. oedematiens* has already been described in Chapter 36.

Each of these organisms, it will be remembered, forms true exotoxins (Ball and Pritchett 1917a, b, Report 1919, Weinberg and Séguin 1918), which apparently possess hæmolytic, leucocidal, and necrotic powers (Kamen 1904, Eisenberg 1907, Simonds 1915, Weinberg and Séguin 1918, Report 1919, Weinberg and Ginsbourg 1927). The most potent toxin is formed by *Cl. oedematiens*; indeed the disease associated with this organism partakes more of an intoxication than a true infection.

Mode of Development of Gas Gangrene in Man.

We have already seen that the injection of a broth culture of *Cl. welchii* or *Cl. septicum* into a guinea-pig will give rise to a disease simulating gas gangrene. Organisms washed free of toxin, or saline suspensions of agar-slope cultures, are non-pathogenic except in very large doses. But when to the toxin-free bacilli a sub-lethal dose of toxin (broth filtrate) is added, the mixture proves intensely virulent. A protocol from De Kruif and Bollman's paper (1917) illustrates the point that a non-lethal dose of filtrate decreases the M.L.D. of the washed bacilli over a thousand-fold.

tendency to form chains or filaments. The spores are subterminal and oval, swelling the vegetative cell in which they occur. Sporulation is often preceded by a marked swelling of the vegetative cell. Deep agar colonies are minute, compact and downy. On the surface of blood agar well separated colonies are flat, round or leaf-like, and hemolytic.

Physiology. This bacillus is a strict anaerobe and, like *Cl. sporogenes*, grows at temperatures as high as 50° C., though the optimum is 37° C. It will grow on the usual laboratory media but is best cultivated in meat or brain medium. These are never discolored nor digested by pure cultures, but they may be slightly softened. Gelatin is liquefied, but coagulated serum and egg are not. Hydrogen sulfide is produced, but indol is not formed and nitrates are not reduced to nitrites. Dextrose, levulose, galactose, maltose, sucrose and



Fig. 134. *Clostridium chauveii* from pure culture. Subterminal oval spores are apparent note the swollen pre-sporulating cell on the far right. Fuchsin, $\times 1050$.

lactose are fermented with the formation of acid and gas, inulin, salicin, mannitol, dulcitol and glycerol are not fermented. It may be noted again that this microorganism may be differentiated from the closely related *Cl. v. puticum* on the basis of the sucrose and salicin fermentation. Litmus-milk cultures become acid and the casein is precipitated, but peptonization does not occur.

Pathogenicity. Natural infections due to *Cl. chauveii* occur principally in cattle. There are still several obscure features in the epidemiology. The disease occurs at special seasons of the year, is connected with certain localities, and is said to show a distinct predilection for the best young stock. The portal of entry is uncertain; whether it enters by way of minute abrasions on the skin or through the gastro-intestinal mucosa is quite unknown. Experimentally, *Cl. chauveii* is pathogenic for cattle, sheep, goats, guinea pigs and mice; horses, asses, hogs, rabbits, rats and pigeons are somewhat refractory.

The symptoms in animals consist in crepitant localized swellings, which in natural infections occur on the thighs, neck or shoulders. The animals become stupid, feverish and anorectic. Treatment is rarely successful, and sick animals usually die in one to two days. The mechanism of the disease is that of progressive bacteremia. A weak exotoxin is produced.

is produced by about half of the toxigenic strains (Robertson and Keppie 1941, Keppie and Robertson 1944, McClean *et al.* 1943, Kass *et al.* 1945, Evans 1945a). Collagenase production, like α -toxigenicity, is also associated with virulence in *Cl. welchii* (Evans 1947a). Simple association, however, is not proof of a causal linkage. The mimicry of the natural lesion by toxic filtrates containing several active components, noted above, is a better proof; but when more stringent immunological evidence (c) is sought, the result is meagre. Evans (1943a, b, 1947b) tested the protection afforded to guinea-pigs by antibodies against α - and θ -toxin, hyaluronidase and collagenase; and found that of the four, only α -antitoxin was protective. The efficacy of active immunization by α -toxoid reinforces this view of the predominating importance of α -toxin, and the subsidiary part, if any, played by the hyaluronidase and collagenase in the genesis of gas gangrene.

may be one of the reasons for the success of early surgical treatment in gas gangrene. The effect of such relief is well illustrated by Evans, who in the guinea-pig made a simple incision in 4-hour-old *Cl. welchii* lesions of the thigh muscles, and recorded that 13 of 18 animals survived, whereas 18 of 18 control animals not so treated died within 3 days (Hartley and Evans 1946).

The rôle of the lecithinase in the muscle lesions is more readily understood than in the toxæmia of gas gangrene. Type A filtrates have an acute action on heart muscle (see Nicholson 1934-5, Kellaway *et al.* 1940); the isolated heart perfused with α -toxin is killed, with the liberation of phosphoryl choline (see Wright 1950), suggesting a direct action of lecithinase. Moreover, not only can α -toxin be detoxified by mixing with lecithin before injection (Wright and Hopkins 1946), but emulsions of lecithin, extracted from human animal tissue, injected into the blood of dogs and mice, protect them against the toxæmia (Zamecnik *et al.* 1945). On the other hand, MacLennan and Macfarlane (1945, Macfarlane and MacLennan 1945) question the conclusion that the generalized toxæmia is directly due to α -toxin, mainly on the grounds that even the early establishment in the blood of high concentrations of antitoxin fixed to muscle: that intoxication does not necessarily produce toxæmia; tissues of intoxicated man of tissue injury secondary in traumatic shock. (See t necessest blood or products sample,

The separation of the toxic components of *Cl. oedematiens* and *Cl. septicum* has not yet progressed to the point where their *in vivo* effects can be profitably analysed, even to the limited extent realized with those in *Cl. welchii*.

"The disease begins, not when a wound has become infected with the pathogenic anaerobes, but from the moment when a group of these bacteria have been enabled to surround themselves with a toxin sufficiently concentrated to abolish the local defences of the tissues" (Report 1919). Once this has occurred, the organisms multiply, produce more toxin, and rapidly invade the tissues. Aside from their aggressive action in the infected tissues, we are largely ignorant of the mode of action of the various clostridial toxins in man.

Diagnosis.

It cannot be too strongly emphasized that the diagnosis of gas gangrene must primarily be made upon clinical grounds. As the figures on p. 1938 show, the

Immunization. A method of prophylactic inoculation which was devised by Arloing, Cornevin and Thomas has been widely used in active immunization. The Lyons⁵¹ vaccine, as distributed by the Bureau of Animal Industry of the United States Department of Agriculture, is prepared as follows. The muscle tissue from a fresh blackleg tumor is pulverized in a mortar, extracted with water, and the extract dried at 35° C. The dry brown scale which results is suspended in water (2 parts), heated for six hours at 95° to 99° C., and injected in appropriate quantities as determined by test and specified on each package distributed. The dried material retains a high degree of activity for several years. Some commercial firms distribute this vaccine in the form of strings to be sewn into the flesh; other dispense the powdered vaccine in the form of pellets which are inoculated by means of a "pill gun." In 1901-2 (July 1 to June 30) 565,628 cattle were vaccinated in the United States. During the previous season 14,817 deaths had occurred, in a similar period after vaccination the number of deaths was only 2902.

Bacteria-free filtrates of edema fluid expressed from the flesh of guinea pigs dead of blackleg or of liquid cultures have been used as immunizing agents with promising results.⁵²

Agglutinating antisera may be prepared against *Cl. chauvei*, and this species appears to be immunologically homogeneous.

CLOSTRIDIUM BOTULINUM

The type of food poisoning termed botulism has been discussed elsewhere (p. 272) and need not be considered further here. In Germany botulism was first definitely observed in 1785 and was and is associated, though by no means exclusively, with the consumption of sausages; hence the not altogether appropriate name botulism (Lat., *botulus*, sausage). The causative bacterium was isolated by van Ermengem in 1896 and named *Bacillus botulinus*. It is now known as *Clostridium botulinum*.

Morphology. *Cl. botulinum* is a large, pleomorphic, gram positive, motile, sporulating rod, 4 to 6 μ in length and 0.9 to 1.2 μ in breadth. The cells occur singly, in pairs and in chains. There are 4 to 8 peritrichal flagella. The spores are subterminal and oval and distend the vegetative cells containing them. Spore formation is variable from strain to strain, some strains producing spores abundantly, others sparsely, but in general spore formation is best in sugar-free media.

Deep agar colonies are translucent, globular and diffuse, or flat and heart-shaped or disc-shaped, according to the consistency of the medium. Surface colonies are relatively large, 5 to 10 mm. in diameter, glistening, translucent at the edges with a thicker brownish center, filamentous, and hemolytic on blood agar.

Physiology. *Cl. botulinum* may be grown on the usual laboratory media under strict anaerobic conditions, cultivation on synthetic solutions has indicated that the amino acids cystine, leucine, lysine, glycine and proline are required.⁵⁴ Amino acids are decomposed by coupled oxidation reduction reac-

1919, 75, p. 4. The preparation of culture. Agr. Res., 1918, 14 253.

suffering from gas gangrene, with mixtures of these sera suggested that both procedures were beneficial (Weinberg and Séguin 1918, Report 1919).

In experimental gas gangrene, both antitoxins and antibacterial sera containing O antibody are protective. But since none of the chief gas-gangrene clostridia is antigenically homogeneous, the efficacy of an antibacterial serum is confined to infections with a given serological type (see Robertson and Felix 1930, Henderson 1934, 1935). It is clearly impracticable to maintain antibacterial sera specific for the multiplicity of serological types. On the other hand the toxic antigens in culture filtrates of these clostridia, though they vary in the content of different components, are to a large extent species-specific, and for routine therapy a mixture of antitoxins can therefore be used. The antitoxin appears to diminish the toxæmia, and thus, by lifting the burden of intoxication from the tissues, makes effective the antibacterial mechanisms of the host (see, e.g., Stewart 1943a, Evans 1945b).

Several different methods have been employed in the standardization of antiserum against gas gangrene (see Dalling *et al.* 1928, Buttle and Trevan 1928, Glenny, Llewellyn-Jones, and Mason 1931, Weinberg, Davesne, and Prévot 1932, Bengtson 1934, Hartley and White 1935, Walbum and Reymann 1935, Ssilanowa 1935, Weinberg and Guillaumie 1938, Ipsen 1939, Ramon 1940, Guillaumie 1941, 1942, 1943, Nagler 1941, Seal and Stewart 1941, Stewart 1945). The two chief methods used for titrating the antitoxin content are intramuscular or intravenous inoculation of mice, and intracutaneous inoculation of guinea-pigs or rabbits, with toxin-antitoxin mixtures. Other methods include flocculation tests analogous to the Ramon titration of diphtheria antitoxin, and, with *Cl. welchii*, neutralization of the lecithinase activity of the α -toxin. International standards exist for *Cl. welchii* (see Hartley 1931), *Cl. oedematiens*, *Cl. septicum* (Hartley and White 1931), *Cl. histolyticum* (Jensen 1936) and *Cl. sordellii* (Bengtson and Ipsen 1939) antitoxins (See also Hartley and Evans 1942-43.) No international action has yet been taken to define these antitoxic sera in terms of the multiplicity of toxins; it may be assumed that they are mainly active against the chief lethal toxin.

During the 1939-45 war, the dose of polyvalent antitoxin recommended for prophylaxis in the British Army was 9,000 units of *welchii* antitoxin, 4,500 units of *septicum* antitoxin and 3,000 units of *oedematiens* antitoxin, to be given intravenously if possible, otherwise intramuscularly. For treatment, three times this dose was recommended, to be given intravenously and repeated 4-6 hourly, according to the response of the patient (see Memorandum 1943). The value of prophylaxis in seriously wounded soldiers cannot at present be estimated. The evaluation of antitoxin therapy of established gas gangrene is complicated by the wide variations in the severity of the disease encountered in the field, in the surgical treatment, and in the type of chemotherapy, local and systemic, often combined with antitoxin treatment.

The British figures for the African and Italian campaigns (MacLennan 1943, 1944a, Macfarlane 1943, MacLennan and Macfarlane 1944, Macfarlane 1945) suggest that the death rates in established gas gangrene are significantly lowered by large total doses of antitoxin (50,000 units or more). Thus in one series 84 per cent. of 25 untreated patients died, but only 51.5 per cent. of 114 antitoxin-treated patients. The antitoxin was effective only in patients receiving adequate surgical treatment. Neither antitoxin nor chemotherapy appears to have much effect on the case-fatality rates when excision of the affected part is incomplete (see also MacLennan and Macfarlane 1944). Occasional striking successes for antitoxin are recorded (Patterson *et al.* 1945).

Chemotherapy of Gas Gangrene.—As early as 1937, Bohlman reported the curative action of sulphanilamide in three cases of gas gangrene. Since that time the sulphonamides

tions rather than by direct oxidation.⁵⁷ Brain, meat and coagulated protein media are blackened and digested, gelatin is liquefied. Milk is peptonized. Hydrogen sulfide is produced, but nitrates are not reduced to nitrites and indol is not formed. Dextrose, levulose and maltose are fermented, the fermentation of other sugars is variable from strain to strain and type to type. The spores are highly resistant and withstand boiling for thirty minutes to twenty-two hours, and autoclaving at 120° for as long as twenty minutes.

Irrespective of the presence of fermentable sugar, a potent soluble toxin is produced which resembles other soluble toxins in most respects. It is, however, unusually stable to heat; heating to 80° C. for thirty minutes or boiling for ten minutes is required to destroy it. Botulinum toxin is unique in that it is not destroyed by the digestive enzymes of the gastro-intestinal tract and hence is

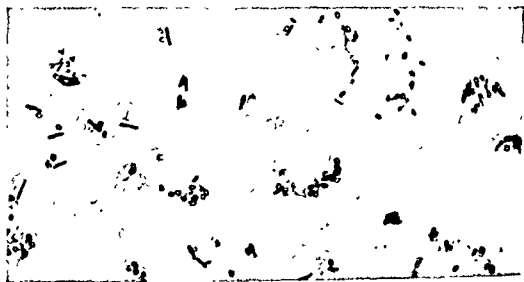


Fig 135. *Clostridium botulinum* Type A from pure culture. Note the subterminal swollen spores and free unstained spores admixed with the vegetative cells. Fuchsin. $\times 1050$.

effective when given by mouth. It is the most potent bacterial toxin known; the guinea-pig MLD may be as small as 1×10^{-6} ml. of broth culture.

Types. *Cl. botulinum* is subdivided into a number of types which differ from one another in that their toxins are immunologically different. The best known of these are Types A and B, which, in the past, have been regarded as solely responsible for human botulism. It is supposed that van Ermengem's original culture, now no longer available, was Type B.

Additional types have been described since the early 1920's. A toxin-producing anaerobic bacillus isolated from fly larvae (the ingestion of which was associated with a paralytic disease of chickens) has been designated Type C.⁵⁸ A closely related bacillus was isolated by Seddon⁵⁹ from botulism of cattle in Australia which he designated *Bacillus parabotulinus*. This bacillus is now designated *Cl. botulinum* Type C β and the fly larvae bacillus *Cl. botulinum* Type C α . The toxins of these subtypes of Type C are related in that C α anti

⁵⁷ Clifton Jour. Bact., 1940, 39,485.

⁵⁸ Bengtson Pub. Health Repts., 1923, 38:340.

⁵⁹ Seddon. Jour. Comp. Path. and Therap., 1922, 35:147, 275.

acute obstruction of the intestine or acute appendicitis has long been a matter of debate. Williams (1926) obtained evidence suggesting that the toxæmia was due to the formation of *Cl. welchii* toxin in the lumen of the obstructed small intestine, and that antitoxin was beneficial in the treatment of such cases. Paralytic ileus following acute inflammation in the abdominal cavity appeared to have the same effect as mechanical obstruction in the genesis of toxæmia; clostridial infection of the inflamed tissue was not observed. Weinberg (Weinberg *et al.* 1928b, Weinberg 1929), however, observed that clostridia could be obtained from 30 per cent. of acute lesions of the appendix (subsequent observers have recorded higher figures); and he tested the effect of antisera prepared against the gas-gangrene organisms in the treatment of conditions associated with an anaerobic invasion of the tissues (Weinberg and Howard 1927, Weinberg *et al.* 1928a). At most, it can be said that such antisera appear to reduce the case-fatality rates, especially when used in conjunction with surgical treatment (for later references see Priestly and McCormack 1936).

Bower, Burns and Mingle (1938) induced a fatal peritonitis in dogs by ligation of the appendiceal vessels, followed by a purgative dose of castor oil; *Cl. welchii* antitoxin reduced the mortality, and prolonged the lives of animals that eventually succumbed to the infection. Normal horse serum was likewise protective, but to a less extent—an effect attributed to the natural antitoxins in the serum. Prophylactic immunization with *Cl. welchii* toxin also reduced the death rate from this experimental disease (Mingle *et al.* 1938). The active participation of *Cl. welchii* may also be inferred from the fact that an antitoxin response is demonstrable in patients with appendicitis. Thus, Bower, Mingle and Paxson (1938) found that 22.2 per cent. of patients with acute appendicitis, 46.6 per cent. of those with active or quiescent pelvic peritonitis, and 69.0 per cent. of those with septic peritonitis after perforation, had more than 1 unit of *Cl. welchii* antitoxin per ml. of circulating blood. The corresponding figure in control patients was 0.0 per cent.

There is little evidence that clostridia, as distinct from other intestinal bacilli, have any significant rôle in the development of hæmorrhagic shock (Fine 1952).

A human case of necrotic hepatitis due to *Cl. œdematians*, resembling braxy of sheep (see below), is reported in man (Mollaret *et al.* 1948).

Enteritis necroticans ("Darmbrand").—We describe below a number of well-recognized intestinal infections of animals due to clostridia. No such disease in man was recognized until 1916, when a severe and often fatal enteritis was described in Germany, characterized by a diffuse sloughing enteritis of the jejunum, ileum and colon. The lesions contained many bacteria, including large Gram-positive bacilli. Zeissler and Rassfeld-Sternberg (1949) isolated from the lesion a type of *Cl. welchii*, subsequently designated Type F by Oakley (1949, see Chapter 36), in which α - and γ -toxins predominated. Type F is found in a small proportion of normal stools, and is said to induce enteritis in guinea-pigs when given intraduodenally, but not by mouth (Schutz 1949). In man, the organism is apparently ingested with food. The disease attacks mainly infants and persons over 30; children and adolescents are more resistant. The predisposing factors are not understood, though dietary imbalance is suspected to be one of them. A viral cause, in addition to the clostridial, is suspected by some (Dieckmann 1949, Hain 1949, Schutz 1949, Zeissler 1949, Hansen *et al.* 1949, Marcuse and Kong 1950).

CLOSTRIDIAL INFECTIONS OF ANIMALS

Blackleg and Gas Gangrene

SYNONYMS: Symptomatic anthrax, quarter-evil, blackquarter.

French: *Charbon symptomatique*. German: *Rauschbrand*.

Blackleg is a disease attacking cattle, sheep, and less often swine (Meyer 1915). It is characterized by the appearance of a crepitant, fluctuating swelling, generally on one of the quarters, followed rapidly by death. Calves are resistant for the

toxin protects against both $C\alpha$ and $C\beta$ toxin, but $C\beta$ antitoxin protects against $C\beta$ toxin but not $C\alpha$ toxin.

A South African strain first described by Theiler and Robinson,⁶⁰ designated by them *Clostridium parabolulinum equi*, has been further studied by Meyer and Gunnison⁶¹ and given the name *Clostridium botulinum* Type D, for its toxin is not neutralized by the antitoxins of Types A, B or C. Still another type, *Clostridium botulinum* Type E, has been isolated⁶² in Russia from fish, consumption of which resulted in human botulism. The Type E toxin is not neutralized by antitoxins of the other four types. There appear to be some strain differences in the action of Type E toxins though complete cross neutralization occurs.⁶³ Type E, it may be noted, has been found as a cause of human botulism in the United States.⁶⁴

Toxins. As just indicated, a number of immunologically different toxins are produced by *Cl. botulinum* and a given toxin is produced by a single type rather than the various toxins being distributed among the types as in the case of *Cl. welchii*. Furthermore, the pharmacological action of the toxins is essentially the same and differentiation can be made only on an immunological basis, i.e., the passive protection test in which a series of experimental animals is passively immunized, each with a single antitoxin, and all challenged with the unknown toxin.

Of these toxins, that of Type A is the most potent, exceeds in toxicity the other soluble toxins such as those of the diphtheria and tetanus bacilli, and is the most potent toxic substance known. The botulinum toxins, though protein in nature, are relatively resistant when given by mouth. The T by Lamanna, McElroy and by alcoholic precipitation in the cold in the first instance, and by sodium sulfate and acid precipitation in the second. The preparations are apparently pure protein with the properties of a globulin and a molecular weight of 9 to 11×10^5 . The LD_{50} dose for the mouse contains 4.5×10^{-9} mg of nitrogen and consists of about 2.1×10^7 molecules. Type B toxin has also been prepared by Lamanna and Glassman⁶⁷ as a pure homogeneous protein but has not been crystallized. It is somewhat less potent than Type A toxin and the mouse LD_{50} dose contains 5 to 9×10^{-9} mg nitrogen. Both pure and crude botulinum toxins may be detoxified with formaldehyde to give formal toxoid which may be used for active immunization.

by
As

⁶⁰ Theiler and Robinson: *Rev. Gen. de Med. Vet.*, 1927, 36:193.

⁶¹ Meyer and Gunnison: *Jour. Inf. Dis.*, 1929, 45:106.

⁶² Cf. Gunnison, Cummings and Meyer: *Proc. Soc. Exp. Biol. Med.*, 1936, 35:278, Kushnir, Brun and Paikina: *Ztschr. Microbiol. Epidemiol. Immunitatf. (U.S.S.R.)*,

Ann., 1941, 117:22.

⁶⁴ Abrams, Keyes and Hottle: *Jour. Biol. Chem.*, 1946, 164:63.

⁶⁷ Lamanna and Glassman: *Jour. Bact.*, 1947, 54:575.

cultures is produced in sheep by the subcutaneous inoculation of solutions of the heat-stable antigen (Mason 1936). Antitoxins are less effective against the bacterial invasion. However, sera for therapeutic use have been prepared against *Cl. chauvæi* and *Cl. septicum*, and their intravenous administration in cases of the fully developed disease appears at times to have given favourable results.

Braxy

SYNONYMS: *Bradsot* = quick plague.

Braxy is the term used for a disease of sheep occurring mainly in the north-western part of Europe. Recent work has rendered it clear that more than one type of disease is called by this name. For descriptive purposes we propose to reserve the term braxy for the type of disease characterized by inflammation of the fourth stomach, and to refer to the so-called German braxy by the term black disease or infectious necrotic hepatitis.

The classical or northern braxy occurs on the western coast of Norway, in Iceland, the Faroe islands, and Scotland. Sheep are most susceptible during their first year. The disease breaks out in the late autumn and early winter months when the animals descend from the hills to the pastures of the lowlands. In Scotland the losses from braxy in some districts may amount to as much as 35 per cent. of the first year's sheep (Gaiger 1924). Clinically, in the usual acute form of the disease, death occurs before any symptoms have been noticed; in the subacute form there is weakness, followed by coma, dyspnoea, and death. The duration of the disease is a matter of a few hours. *Post mortem*, there is severe inflammation of the fourth stomach and duodenum, with œdema and hæmorrhage—sometimes necrosis—of the mucosa and submucous tissue. The peritoneal fluid is often turbid and in excess; there are degenerative changes in the visceral organs, and an acute parenchymatous nephritis.

The causative organism was described by Nielsen in 1888, who found it in large numbers in the stomach wall. It was identified by Gaiger (1922) with *Cl. septicum*. The mode of infection is obscure; experimental feeding with pure cultures of the bacillus apparently fails to reproduce the disease; Jensen was successful only 5 times in 1,545 attempts (Gaiger 1924). Subcutaneous injection produces black-leg, *post mortem*, lesions may be present in the abomasum simulating those of the natural disease (Hamilton 1902, 1906); there is no evidence however that natural infection occurs by this route. Again we are forced to conclude that some accessory factor must be present before the bacillus is able to invade the tissues. Experimental work on guinea-pigs by Borthwick (1934) suggests that intestinal stasis and exposure of the animal to cold are important factors in determining the invasion of the tissues by *Cl. septicum*. Though the feeding of cultures to normal guinea-pigs rarely set up infection, inhibition of peristalsis by narcotine, or preliminary exposure to cold, resulted in the development of a fatal infection in a fairly high proportion of the animals.

A considerable degree of protection is afforded by the use of Jensen's sero-vaccine.

Gaiger (1922) obtained promising results by the use of a sterile filtrate of a culture of *Cl. septicum*. Of 10,340 hogs (1st year sheep) vaccinated, 2.39 per cent. died of braxy, whereas among 3,800 control animals the mortality from braxy was 9 per cent. (see Gordon 1934a).

proteolytic (sometimes designated ovolytic but digesting proteins other than coagulated egg albumin), whose cultural characteristics have been described above, and the other saccharolytic or fermentative in character, whose members do not hydrolyze coagulated native proteins. The proteolytic group includes Type A and some strains of Type B (the majority of the American Type B strains are proteolytic, while a great many of the European Type B strains are not). The non-proteolytic group includes some strains of Type B, and, so far as is known, all strains of Types C, D and E. Bengtson⁶⁸ has suggested that only the non-proteolytic varieties be designated *Cl. botulinum* and that the proteolytic varieties be termed *Cl. parabotulinum* (because van Ermengem's original strain was nonproteolytic)—a suggestion that has been adopted in the Bergey classification. It would appear undesirable, however, to designate practically all of the bacilli causing human botulism in the United States as "parabotulinum."

TYPES OF CLOSTRIDIUM BOTULINUM

Type	Synonym	Biochemical Character	Disease	Antitoxin
A		Proteolytic	Botulism of man, limberneck of chickens	Specific
B		Some strains proteolytic	Botulism of man, limberneck of chickens	Specific
Ca	Fly larvae bacillus	Non-proteolytic	Paralytic disease of chickens, botulism of wild ducks	Neutralizes C β toxin
C β	<i>Cl. parabotulinum</i>	Non-proteolytic	Forage poisoning of cattle (Australia)	Specific
D	<i>Cl. parabotulinum equi</i>	Non-proteolytic	Lamziekte of cattle (Africa)	Specific
E		Non-proteolytic	Botulism of man	Specific

Pathogenicity for Man. Human botulism is invariably the result of eating preserved foods in which the bacillus has grown and produced toxin. In Europe most cases have been due to the consumption of various kinds of preserved meats, such as sausage, ham, potted goose or duck and the like, while in the United States the incriminated foods have been canned vegetables for the most part. There are surprisingly of the ubiquitous distribution of been found most commonly in the Rocky Mountain and Pacific Coast states, while Type B predominates in the Mississippi Valley, Great Lakes region and Atlantic Coast states. Type A predominates in English soils, though Type B may be found also. From 1899 to 1935 there were 261 reported cases of

⁶⁸ Bengtson U. S. Pub. Health Ser. Hyg. Lab. Bull. No. 136, 1924.

experiments, Turner was able to show that in black disease the necessary conditions for growth of the causative organism were found in the necrotic areas produced by invasion of the liver with *Cl. welchii*. In these areas the *Cl. welchii* was responsible for the main part of the damage. It was found to be latently infected with the spores of *Cl. adematensis*, but not until suitable necrotic foci were established by invasion with liver flukes did the infection become active.

Consequent on this work Turner prepared a formalized broth vaccine with which very satisfactory results were obtained in field trials. The figures showed that when two doses were given, the mortality from black disease was reduced on an average by about half, as compared with control animals on the same farm. Even more favourable results attended the use of three doses of vaccine.

Vaccination of over 270,000 sheep in Victoria, Australia, during the years 1933-36 was accompanied by a reduction in the annual mortality due to black disease from 4-15 per cent. to 0.02-0.05 per cent. (Wardle 1936; see also Rose 1936). According to Oxer (1937) alum-precipitated vaccine is a more effective immunizing antigen.

Jamieson (1949) showed that in northern Scotland the disease was caused by *Cl. adematensis* Type B, which was also present in about 1 per cent. of livers of healthy sheep and cattle in areas free from black disease, and in about 17 per cent. of livers of healthy animals in black disease areas. He was able to activate latent disease in infected guinea-pigs, but not rabbits, by the oral administration of cercariae of *F. hepatica*.

Osteomyelitis of Buffaloes.—The buffaloes of Indonesia are said to suffer from a non-fatal osteomyelitis, which particularly affects the humerus and femur. The causative organism is indistinguishable from *Cl. gigas*, except that it is non-pathogenic to laboratory animals. The pathogenesis of the disease is obscure; and inoculation of the buffalo with the organism is not followed by osteomyelitis (Kranefeld 1930, Kranefeld and Djaenoeidin 1933).

Infections due to *Cl. welchii*: Lamb dysentery; "Struck"; infectious enterotoxaemia; pulpy kidney disease

A number of acute toxæmic diseases have been described in different parts of the world, mostly affecting sheep, in which the lesions appear to result from an intestinal infection with a variety of *Cl. welchii*. The pathogenesis of these diseases is to a large extent obscure, but considerable progress has been made in their control by vaccination and serotherapy.

Lamb dysentery is a disease that takes a heavy toll of life among the lambs during their first 2 weeks of life (Gaiger and Dalling 1921). It is particularly prevalent in the border counties of England and Scotland. Pathologically it is characterized by an enteritis varying from a mild congestion of the intestinal mucosa to a condition in which extensive tracts of the small and large intestine become necrosed and ulcerated. On the surface of the inflamed mucosa and ulcerated areas a bacillus is found closely allied to *Cl. welchii*, but differing from it in certain cultural particulars and in its production of a more powerful toxin (Dalling *et al.* 1925, Dalling 1928b, 1931-32). This organism is sometimes referred to as the lamb dysentery bacillus, and sometimes as the *agni* variety of *Cl. welchii*. It is at present probably better to call it *Cl. welchii* Type B (see Chapter 36). The organ-

botulism in the United States, the greatest numbers in any one year being 23 in 1922 and 22 in 1935. Over that period of time 101 cases were reported from California, by far the greatest number in any single state.

As in the case of the other sporulating anaerobes, the disease produced by *Cl. botulinum* is an intoxication, in botulism, in fact, there is no invasion of the tissues and the toxin is preformed outside the body. Under experimental conditions in which massive doses of spores have been injected, it is probable that no infection has been set up. *Cl. botulinum* has, however, been found in three cases of contaminated wounds in mixed cultures with aerobic and anaerobic bacteria, but no symptoms of botulism were apparent.⁶⁹ Under rare circumstances, then, it may proliferate in the tissues.

Until the recent reports of the occurrence of Type E, Types A and B were the only types of *Cl. botulinum* involved in human botulism. The pharmacological activities of these toxins are substantially identical, the effect appears, in the main, to be exerted on the peripheral nerve endings, possibly those of the autonomic system, and paralysis of the motor nerve end plates in the striated muscles and the diaphragm results. Whether there are pathological changes in the nerve cells of the central nervous system, such as degeneration of the Nissl bodies, is uncertain. The symptoms include vomiting, constipation, ocular paresis and pharyngeal paralysis. Death may occur within a day of the onset of symptoms or may be delayed for as long as a week. At autopsy the liver, kidneys and meninges are congested and there may be thrombosis. The case fatality is variable, in the United States it has been 60 to 70 per cent, but in Germany it is much lower, perhaps 25 per cent.

Pathogenicity for Lower Animals. Associated with human cases of botulism there have been numerous outbreaks of limberneck, a paralytic disease, among fowls fed the toxin containing food. Other forms of botulism in lower animals occur under natural conditions, however. *Cl. botulinum* Types C and D appear to be associated exclusively with the disease in lower animals. Certain forms of forage poisoning in cattle and horses in Australia are botulism, but whether the bacilli grow and form toxin in the fodder or whether the disease results from the ingestion of rabbit carrion is not entirely clear. The South African disease of cattle, lamziekte, is botulism resulting from the ingestion of contaminated carrion. In the United States botulism of wild ducks and other waterfowl due to Type Ca is prevalent and causes the death of thousands of ducks each year.⁷⁰ The source of the toxin ingested by these fowl is uncertain.

Experimentally rabbits, guinea pigs, mice, monkeys, cats and dogs are susceptible to toxin administered parenterally or *per os*. The symptoms are similar to those of naturally infected animals and of man, and the postmortem findings are much the same. Experimental animals vary widely in their susceptibility to the toxins of the various types of *Cl. botulinum*.

Immunity. Formol toxoid may be used as an immunizing antigen to produce an active immunity with circulating antitoxin present in the blood. Such active immunization has been carried out in lower animals when eco-

⁶⁹ Hall Jour. Bact., 1945, 50 213

⁷⁰ Cf. Gunnison and Coleman Jour. Inf. Dis., 1932, 51:542. See also Kalmbach, U. S. Dept. Agr., Bur. Biol. Surv. Wildlife Res. and Manag. Leaflet BS-120, 1938.

an antitoxin to *Cl. welchii* Type D. His work suggests that this organism may play a part in a number of diseases of sheep, lambs, and perhaps horses.

The conditions governing the formation of a toxin in most of the diseases that we have been discussing are still obscure, and there is a considerable field open for an investigation of the type so successfully undertaken by Turner (1930) in black disease. It is interesting to reflect that whereas anaerobic infections in man and the carnivora are generally preceded by trauma, in herbivora they often occur spontaneously (Heller 1920).

The genesis of enterotoxæmia affords an illustration of the complexity of the problem. The main clinical and pathological findings in this disease appear to be the result of intoxication by the ϵ -toxin (Nicholson 1934-35, Kellaway, Trethewie and Turner 1940, Gordon *et al.* 1940). In 1935 Bosworth and Glover observed that the lethal power of the toxin of *Cl. welchii* Type D, but not of Types A, B or C, was increased when added to an intestinal filtrate of a normal sheep, guinea-pig or rabbit. But in spite of the increase, exactly the same amount of antitoxin was required to neutralize the toxin, whether it was free or mixed with the intestinal filtrate. These workers suggested that the toxin might form a highly toxic complex with the intestinal tryptic ferments, possessing some of the properties of histamine. More recently Turner and Rodwell (1943) have reported that all kinds of proteolytic enzymes, including those of *Cl. welchii* itself, can "activate" the ϵ -toxin; they postulate an ϵ -prototoxin which may be converted by any of these enzymes into ϵ -toxin. As regards the conditions for toxin formation in the gut, Roberts (1938) concluded from *in vitro* and *in vivo* experiments that in milk-fed lambs the acidity of the abomasum contents normally prevented any proliferation of *Cl. welchii*. When infective material is present after the ingestion of an abnormally large feed, the acidity is depressed by the casein in the feed, and *Cl. welchii* Type D may proliferate, producing a toxin which is activated in the small intestine. Bullen (1952), however, showed that many sheep have *Cl. welchii* Type D in some part of the alimentary tract, and he considered the means whereby such an endogenous source of infection remained harmless in healthy sheep. Using animals with permanent fistulæ in the rumen, duodenum or ileum, Bullen, Scarisbrick and Maddock (1953) observed that spores of the organism introduced into the rumen were rapidly destroyed, but that those which survived to reach the intestine multiplied there for a short period, producing considerable quantities of ϵ -toxin, and then disappeared. In the normal animal, any pathogenic accumulation of either bacilli or toxin in the intestine, however, appeared to be counteracted by the rapid flow of the intestinal contents.

The source of infection is presumably *Cl. welchii* Type D in the soil, though it is found there only occasionally, Type A or non-toxic *Cl. welchii* being the predominating varieties (see Taylor and Gordon 1940). However, it is known that Type D strains readily lose their power to produce ϵ -toxin on subculture (Borthwick 1937), and it is possible that many of the Type A strains found in soils and the intestinal content of animals may be degraded Type D strains.

(For an account of the anaerobic toxæmias of animals, see Woodruff 1936.)

For botulism in animals see Chapter 72

nomically feasible; in Australia botulism of sheep and cattle has assumed sufficient proportions to justify such active immunization, and it has been applied on a small scale.⁷¹ Man may also be immunized with fluid or alum-precipitated toxoid of Type A or Type B or a mixture of both types. Toxoid may be given in four doses at two week intervals or three doses at three to four week intervals; an arbitrarily defined protection level of 0.02 units of antitoxin per ml. of circulating blood is reached in 50 per cent of those inoculated in about three months after initiation of the immunization.⁷² Under ordinary circumstances naturally occurring botulism in man is so rare that active immunization is not worth while.

Botulinum toxin is an excellent antigen and high-titer antitoxic sera may be produced. Under experimental conditions these antitoxins have marked prophylactic value, but their therapeutic efficacy is slight. It may be pointed out that in botulism, as in tetanus, the symptoms are a consequence of the injury to the nerve tissue and the administration of antitoxin serves only to neutralize circulating toxin. The almost complete lack of therapeutic effect of botulinum antitoxin in human botulism is undoubtedly attributable to the inevitable too late administration.

DIFFERENTIATION OF THE SPORULATING ANAEROBES

In many respects the isolation and identification of the sporulating anaerobic bacilli is somewhat more difficult than in the case of the aerobic and facultatively anaerobic bacteria. Primary cultures may be inoculated into deep brain medium and, after incubation, examined for spores, heated to 80° C. for ten minutes, then subcultured. Representative colonies may be picked from shake cultures or from the surface of plates incubated either in an anaerobic jar or in the Spray dish. A procedure for the rapid identification of the anaerobes associated with gaseous gangrene has been given in some detail by Reed and Orr.⁷³

Spray⁷⁴ has divided these bacilli into main groups on the basis of reaction in iron milk⁷⁵ and has developed a key for their further differentiation and identification on the basis of morphology, physiology and pathogenicity. Of no small practical utility, his key is outlined below in an abridged form, the original paper should be consulted for details concerning other anaerobic species and the preparation of the media.

SPRAY'S KEY TO THE SPORULATING ANAEROBES

- I. Iron milk active gaseous fermentation, early coagulation (12–48 hours), no digestion of clot, no blackening.
 Lead acetate, strongly blackened.
 Nitrite +, indol —, gelatin +, motility —.
 Glucose +, lactose +, sucrose +, salicin —.

⁷¹ Cf. Bennetts and Hall. Australian Vet. Jour., 1938, 14:105.

⁷² Nigg, et al. Jour. Immunol., 1947, 55:245; Reames, Kadull, Housewright and Wilson, *ibid.*, 1947, 55:309.

⁷³ Reed and Orr. Proc. Soc. Exp. Biol. Med., 1941, 48:535, War Med, 1941, 1:493

⁷⁴ Spray. Jour. Bact., 1936, 32:135.

⁷⁵ Fresh whole milk is sterilized in deep tubes, each of which contains a 50 × 7 mm. piece of No. 26 gauge black stove-pipe iron.

CHAPTER 79

MISCELLANEOUS DISEASES

NECROBACILLOSIS, OZÆNA, RHINOSCLEROMA, GRANULOMA VENEREUM, SOFT CHANCRE, CAT-SCRATCH FEVER, GLANDULAR FEVER, BARTONELLA INFECTIONS, AND VARIOUS OTHER DISEASES

NECROBACILLOSIS AND RELATED INFECTIONS

THIS term may be used to cover a wide variety of lesions in man and animals due to infection with the non-sporing anaerobic bacillus known as *Fusiformis necrophorus* (see Chapter 18).

In man, infections by organisms of the *F. necrophorus* group, which includes those strains named *B. funduliformis*, seem to be rare. This may result from failure to submit all material from purulent infections to routine anaerobic cultivation. Holicky (1940), for example, recorded that Gram-negative, anaerobic non-sporing rods and fusiform bacilli were obtained, sometimes in pure culture, from about 2 per cent. of over 5,000 specimens from infected patients in a surgical clinic. Judging from the species-frequency observed in human infections, it is probable that the majority of the strains isolated were of *F. necrophorus* type.

The disease may take the form of purulent or gangrenous inflammation of the skin (see Shaw 1933). The mode of infection is not always clear, but a number of cases have followed bites or injuries from the teeth of animals. More commonly it occurs as a deep-seated infection, with purulent abscesses in the abdominal cavity, liver or lung, many of which appear to be the result of a pyæmia. It often complicates operations on appendix abscesses or other septic lesions of the lower gut. The case fatality among patients with well-established infection is over 50 per cent, and up to 80 per cent when there is septicæmia. The infecting organism is presumably derived from the alimentary tract, though *F. necrophorus* has seldom been isolated from normal stools. In some instances, however, *Fusiformis* strains from infective lesions in man resemble those found in normal tissues. Thus, Spaulding and Rettger (1937) demonstrated cultural and serological similarities between strains from the normal and from the infected mouth and vagina, and from lung abscesses. What relation the disease has to Vincent's angina (see Chapter 80), and to the various necrotic diseases of the mouth in which the closely related organism *Fusiformis fusiformis* seems to play a part, is not clear.

It is impracticable to review the types of human infection that have been reported, but we may note the following. Puerperal sepsis and other infections of the female genital tract (Harris and Brown 1927, Delbove and Reynes 1941, Cooper and Robson 1947, Hartl 1950); abdominal, hepatic or lung abscesses (Cunningham 1930, Cohen 1932, Thompson

Clostridium—The Spore-forming Anaerobes

Spores ovoid, abundant, central-excentric, not markedly if at all swelling rod.

(1) Pathogenic

Clostridium sordellii

(2) Non-pathogenic.

Clostridium bifermentens

(*Clostridium centrosporogenes*)

B. Lead acetate: no blackening, no browning.

Nitrite —, indol —, gelatin +, motility +.

(Wine-red color in iron gelatin (24-48 hours).)

Glucose —, lactose —, sucrose —, salicin —.

Microaerophilic.

Spores ovoid, abundant, excentric-subterminal, swelling rod.

Clostridium histolyticum

V. Iron milk. no gaseous fermentation, no digestion, no blackening, coagulation late if any (15-20 days or more).

Lead acetate: not blackened, but showing smoky browning at 24-48 hours, not measurably increased on incubation.

Nitrite —, indol ±, gelatin +, motility +.

Glucose —, lactose —, sucrose —, salicin —.

Spores spherical, not abundant, terminal, swelling rod.

A. Toxic.

Clostridium tetani

B. Non-toxic,

Clostridium putrificum

necrosis of cattle, pigs, and sheep, labial necrosis of rabbits, and a number of other manifestations (see Beveridge 1934).

In calf diphtheria, which was studied by Loeffler (1884), a false membrane stretches from the throat down into the trachea; the superficial parts are caseous and friable, the deeper parts firmly adherent. Microscopically the membrane consists of a superficial layer containing large numbers of micrococci; a middle layer, amorphous and unstained; and a deep layer containing granular detritus with some cells and the characteristic long wavy rows of bacilli; these are separated by a narrow unstained zone from a dense infiltration of cells. The lungs contain pneumonic foci, in which much the same microscopical picture is evident. A similar disease to calf diphtheria is said to occur in lambs.

Foot-rot of sheep was at one time regarded as due to *Fusiformis necrophorus*, but Beveridge (1935, 1941) in Australia cast doubt on this. He found that neither *F. necrophorus* nor *Treponema penorthum*, which could be seen microscopically in smears from the lesions, was able, alone or together, to set up the disease in healthy sheep. On the other hand, a long anaerobic Gram-negative non-sporing rod having knob-like ends, *F. nodosus*, which was present in smears in only small numbers, was cultivated in a medium containing 10 per cent. of horse serum, and when applied in pure culture, or better still with *Trep. penorthum*, gave rise to typical foot-rot in healthy adult Merino sheep. Working with scrapings from lesions, not with pure cultures, Beveridge found that the infecting agent, as judged by its ability to cause foot-rot in healthy sheep when applied to the scarified area between the digits, might survive for a few days in mud or in sheep faeces, but never for as long as 3 weeks. On the other hand it could persist for over 3 years in lesions of chronically infected sheep, and for as long as 7 months in superficial skin lesions between the digits. Sheep, however, that had recovered from the disease for a month or more appeared to be no longer infective. Since the causal agent does not survive for more than a short time under saprophytic conditions, it should be possible to free pastures from infection by removal, preferably during the summer, of all infected sheep.

Labial necrosis of rabbits is characterized by a dark bluish-red discoloration of the under lip, accompanied by a tender swelling. The infiltration passes gradually down the under surface of the mouth and front of the neck, and in about 8 days reaches the upper opening of the thorax. Constitutional symptoms develop about the 5th day; a thin watery discharge comes from the nose; the respirations increase in rate; the temperature rises 1-1.5° C. and the animals die in an emaciated condition with considerable dyspnoea. *Post mortem*, section shows that the under lip is converted into a yellowish-white, compact, bacon-like, necrotic mass, which in places extends to the bone; around the necrotic mass is a reddish-black border. The cervical glands are greatly swollen, juicy, and greyish-red; sometimes they show small caseous foci. There is bloody, slightly turbid fluid in the pleural and pericardial cavities with some fibrin deposit on the surrounding serous membranes. A few pneumonic areas may be seen in the lungs, with yellowish-white streaks passing from the pleura into the dark red pulmonary tissue. The spleen and other viscera appear normal. The disease was first described by Schmorl (1891), who observed an epidemic among his laboratory rabbits.

The diagnosis of necrobacillosis is made by morphological and cultural methods. In the lesions the organisms appear mostly as Gram-negative wavy non-branching filaments lying parallel to one another. Attempts to isolate them may be made directly by the inoculation of sterile media, or by streaking plates of blood or potato extract agar, or by streaking plates of blood or potato extract agar containing gentian violet and incubating anaerobically (8-10 days at 37°C). In view of the extreme oxygen sensitivity reported in some species of *Fusiformis*, surface plates should be subcultured as soon as possible after removal from the anaerobic atmosphere. The incorporation in the plate media of reducing substances like thioglycollic

Chapter 29

THE GLANDERS BACILLUS (*MALLEOMYCES MALLEI*)

Glanders is a disease seen, as a rule, only in the solipeds (horse, mule, ass), but is occasionally transmitted to other domestic animals, to wild animals and to man. Early regarded by many as a spontaneous, non-infectious affection, the transmissibility of glanders was demonstrated in 1837 by Rayer, who infected a horse by inoculating it with material from a case of glanders in a human subject. The causative bacillus was discovered in 1882 by Löffler and Schutz, whose work was soon confirmed and extended by Kitt, Weichselbaum and others.

Morphology and Staining. The glanders bacillus is a small rod, straight or slightly curved, usually with rounded ends, and often of irregular contour. Rather wide variations in size are observed, the average length may be taken as 2 to 5 μ and the average breadth 0.5 to 1 μ . It is often compared to the tubercle bacillus but is usually found to be somewhat broader. In culture the bacilli tend to be shorter and more uniform in size than those observed in pus smears. In pus they are sometimes found within the leucocytes but more often occur free.

The bacilli may be
ends in wh.
non-encapsulated, and do not form spores.

Colonies on agar are small, round, convex and amorphous in consistency. They are translucent and yellowish in color and upon aging (eight to ten days) become more opaque and the center may become light brown. The growth on potato usually exhibits a characteristic appearance, clear, amber, honey-like colonies appear which may coalesce, and frequently the potato

fuchsin). The bacilli are not acid-fast and are gram negative. Cells from young cultures take the stain fairly uniformly, but those in older cultures stain irregularly, with a tendency to bipolar staining. Granules and coccus-like bodies within the cell take the stain somewhat more readily, and the bacilli not infrequently have a beaded appearance in stained preparations. Worley and Young¹ have shown that this irregular staining is due to the presence of lipid

¹ Worley and Young Jour. Bact., 1945, 49:97.

(see Canning and Guerry 1912). It is commoner in females than in males and affects particularly young adults living in rural districts. Multiple cases occasionally occur in one family, but the contagiousness of the disease appears to be very low. It has not been transmitted to animals (Perkins 1907, Gasiorowski 1929, Levine and Hoyt 1918).

The aetiology of the disease is still in doubt. In 1882 von Frisch cultivated a member of the capsulatus group from the lesions; he called it *B. rhinoscleromatis*. By injecting cultures into the submucous tissue of the nasal septum, he produced in rats a small tumour which contained the organisms. Subsequent workers showed that the scleroma bacillus belonged to serological Type C of the capsulatus group, but could be distinguished from Friedländer's bacillus of the same type by its possession of a different O antigen and by its biochemical reactions (see Chapter 28). The organism appears to be seldom found except in association with cases of scleroma; and the fact that the serum of a high proportion of patients and of some contacts gives a positive complement-fixation reaction with it suggests that it may be causally related to the disease (Tomáček 1925, Quast 1926, Gasiorowski 1929, Kouwenaar, Maasland and Wolff 1931, Levine and Hoyt 1917). Streptomycin has been suggested as a method of treatment.

GRANULOMA VENEREUM

SYNONYM: *Granuloma inguinale*

This disease, which is not to be confused with lymphogranuloma inguinale (see p. 2093), is a venereal disease characterized by a slowly progressive ulceration of the tissues in the genital region. It is widespread in the tropics. Both sexes are attacked. The incubation period varies from a few days to 2 or 3 months. Spontaneous cure is uncommon, but the infection is susceptible to treatment with antimony.

Many years ago Donovan (1905) described the constant presence in smears from the ulcerated lesions of characteristic intracellular bodies, which he regarded as parasites, and which now usually bear his name. They are often contained in giant mononuclear cells. They resemble bacilli of the Friedländer group, are Gram-negative and are surrounded by a well-defined capsule, which can be demonstrated by Wright's stain. Non-capsulated forms, however, are also present. In spite of several claims to have cultivated the causative organism, the precise agent responsible for this disease remained obscure till Anderson, DeMonbreun and Goodpasture (1945) succeeded in cultivating it in the yolk sac of the developing chick embryo. The organism will not grow on ordinary media or on the chorio-allantoic membrane. It requires some substance present in the yolk of a fertile egg for its nutrition. After a little adaptation it will grow in yolk removed from the embryo and transferred to a test-tube. Yolk from embryos in the early stages of development (4-8 days) is better than that taken later (12-14 days). The growth-supporting substance in yolk withstands heating at 60° C. for 30 minutes.

Dulaney, Guo and Packer (1948) were successful in growing it on a medium consisting of equal parts of Locke's solution and of egg yolk taken from 5 to 8 day chick embryos, sloped, and heated at 80° C for 15 minutes, and Rake and Oskay (1948) on a modified Levinthal agar. On this medium translucent shiny colonies appeared in 48 hours increasing

granules which do not stain with the usual dyes but may be demonstrated with Sudan black B or iodine-fuchsin.

Physiology. Growth occurs on ordinary nutrient media but is poor and slow on primary isolation. Forty-eight hours' incubation is generally necessary for the appearance on solid media of colonies 0.5 to 1 mm. in diameter. Growth is materially enhanced by the presence of glycerol, but glucose is without effect. A slightly acid reaction is favorable and the optimum temperature is 37° C., though growth may occur over the range from 22° to 44° C. Growth on enriched media such as Löffler's serum medium or horse-blood agar is not markedly better than on glycerol agar.²

The glanders bacillus is quite inactive biochemically. With the exception of glucose, there is no action on the usual carbohydrates, and even the glucose fermentation is irregular and variable from strain to strain. Coagulated serum

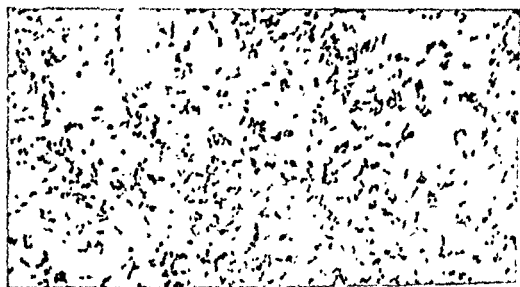


Fig. 136. *Malleomyces mallei* in pure culture. The generally poor staining is apparent and bipolar staining may be observed in some of the cells. Methylene blue, $\times 1250$.

is not digested,
by some strains
is sparse and no

for twenty-six to forty days, the gelatin does not solidify on cooling. Indol is not produced, and nitrates are not reduced to nitrites; small amounts of hydrogen sulfide may be formed. Slight acid production sometimes occurs in milk, with coagulation by the tenth day of incubation and decolorization of the indicator in the lower part of the tube.

The bacillus is but slightly resistant to adverse physical and chemical agents, being readily killed by heat (55° C. for ten minutes) and bactericidal chemicals. Desiccation experiments have not given uniform results, it is said that cultures dried on threads remain viable for three or four weeks. Pure cultures appear to be more resistant to desiccation than the bacilli in the nasal secretions from diseased animals, for infected discharges are usually sterile within

² For recent studies on the morphology and physiology of this organism see Miller et al.: Jour. Bact., 1948, 55-115.

is positive in about two-thirds of the cases. An objection to it is that it may remain positive for years and cause confusion with the Frei test made for the diagnosis of lymphogranuloma inguinale. A positive reaction to one of these tests is of more diagnostic value than a reaction to both (Greenblatt and Sanderson 1937, Dulancy 1937).

Vaccine treatment, and treatment with an antiserum prepared by the inoculation of rams, were used at one time (Reenstierna 1923, Nicolle and Durand 1924, Hababou-Sala 1925, Watanabe 1939), but the usual treatment now is by sulphonamides or one of the antibiotics.

CAT-SCRATCH FEVER

Cat-scratch fever is a disease characterized by a suppurative subacute enlargement of one or more lymph nodes healing spontaneously in the end. The incubation period is about a week. A red papule may appear at the site of the scratch followed a day or two later by swelling and tenderness of the regional lymphatic nodes. Fever may or may not be present and is usually irregular. The lymphatic nodular inflammation may resolve, but more commonly it goes on to suppuration with discharge of pus through the skin and the formation of a fistula that may take weeks or months to heal up. Recovery is gradual. The disease is said to be widespread in the United States of America (Foshay 1952). Cases have been reported in Europe (Debré *et al.* 1950, Campbell and Wheaton 1952) and in Australia (Tonge *et al.* 1953). Other injuries besides those inflicted by a cat may be responsible. In some respects the disease resembles tularæmia. Diagnosis has to be made from tularæmia, rat-bite fever, glandular fever, and *Pasteurella* infection, as well as from tuberculous and mycotic lymphadenitis and from Hodgkin's disease and lymphosarcoma. The blood often shows a polymorphonuclear leucocytosis. A skin test made with pus from a bubo heated to 60° C. for an hour or two on 2 successive days gives rise to a strong reaction reaching its maximum in 24 hours and persisting sometimes for weeks or months. The cause of the disease is unknown but is believed to be a virus (see Greer and Keefer 1951, Lange 1951). Mollaret and his colleagues (1950, 1951) described the successful transmission of the disease to *Cercopithecus* monkeys by intracutaneous inoculation. In both human and monkey material acidophilic granulo-corpuscles resembling those seen in psittacosis can be seen histologically in cells of the affected tissues. It is doubtful, however, whether the virus belongs to the psittacosis-lymphogranuloma group. Patients affected with cat-scratch fever do not react to the Frei test, nor do lymphogranuloma patients react to the cat-scratch fever antigen. Aureomycin is said to be of value in treatment.

ACUTE NON-SPECIFIC MESENTERIC LYMPHADENITIS. EPIDEMIC EPIDIDYMO-ORCHITIS

Reference may be made here to two diseases characterized by inflammation of lymphatic or other glands whose aetiology is completely obscure. Ward-McQuaid (1951) may be consulted for references to the disease acute non-specific mesenteric lymphadenitis, which affects mainly children; and Tunbridge and Gavey (1946) for a description of an outbreak of epidemic epididymo-orchitis occurring among members of the Services in Malta during the summer of 1943.

a few days. Cultures die out in four to six weeks but may be maintained by transfer on glycerol agar.

Classification. The relation of the glanders bacillus to other bacteria is by no means clear. In certain respects, such as the occurrence of branching and the favorable effect of glycerol on growth, it resembles the *Mycobacteria* though it is not acid-fast. It has, in fact, been grouped under the *Actinomycetales* by some workers in the genus *Actinobacillus*. In other respects it is related to the *Brucella* species with which it is grouped in the current Bergey classification. Generally termed *Bacillus mallei*, it was formerly designated *Pfeifferella* by Bergey (4th ed.) as a newly created genus. This generic name has now been discarded and a new one, *Malleomyces*, coined.

Pathogenicity for Lower Animals. Under natural conditions the horse is chiefly affected, but cases are occasionally observed in the carnivora (cats, dogs, menagene animals) and in goats and sheep. Swine and pigeons are slightly susceptible. Cattle and house rats are immune. Rabbits and guinea pigs are somewhat susceptible to experimental inoculation and the hamster and ferret are the most susceptible of the usual experimental animals.²

Glanders manifests itself in an acute and a chronic form, which run into one another, the latter frequently terminating in an acute attack. The acute form is ushered in usually by a chill and the appearance of a high temperature in advance of any local manifestation. In a few days the mucous membrane of the nose is inflamed and becomes studded with nodules, the lymphatic system becomes largely involved, and edematous swellings appear in various parts of the body. General symptoms become more grave, and death follows in from eight to thirty days. The mule, and especially the ass, suffer commonly from the acute disease. The chronic form is the more usual type in the horse (90 per cent of cases). A great variety of symptoms and lesions have been noted in the latter animal, and the disease pursues most diverse courses in different individuals. The nasal membrane is often affected, and there is a profuse and infectious catarrhal discharge. Cutaneous glanders is known by veterinarians as *farcy*, the thickenings of the superficial lymphatics being termed "farcy buds" or "farcy pipes." In all forms of glanders there is a tendency to the production of nodules, which soften and pass over into ulcers. The glanders nodule has been considered by some writers to be structurally similar to the nodule formed by the tubercle bacillus (p. 633) but most observers are agreed that the former is a degenerative rather than a proliferative formation, and that it is radically different from the tubercle.

Experimental inoculation with pure cultures has given positive results not only in the horse, in which the characteristic features of the disease are reproduced, but in guinea pigs, field mice and other small rodents. House mice and white mice show a high but not absolute resistance, in contrast to the great susceptibility of field mice. The guinea pig responds to inoculation in a typical fashion, and has been utilized for differential diagnosis. Both in the natural and in the experimental infection the bacteria are found chiefly in the nasal secretions and in the contents of the young nodules; in the older ulcers they are relatively few in number. The blood, as a rule, contains glanders bacilli only in acute general infection.

² Miller et al.: Jour. Bact. 1948, 55 127.

and to reproduce a similar result in a second monkey inoculated with blood from the first monkey after it had been hæmolyzed and filtered through a Seitz EK disc. The virus was grown in tissue culture, and still proved capable after ten generations of causing a monocytosis in monkeys. Mice inoculated intracerebrally proved insusceptible. Nettleship (1942) described the production of pearl-grey nodules with heavy monocytic cell infiltration in the chorio-allantoic membrane of chick embryos inoculated with Berkefeld filtrates of nasal washings or with whole blood of glandular fever patients. Wising (1942) failed to reproduce the disease in rabbits inoculated intravenously with heparinized blood of febrile patients, but had some success with monkeys. Of seven *Macacus* or *Cercopithecus* monkeys inoculated intracerebrally, intraperitoneally, or subcutaneously with fresh lymph node suspensions from patients in the acute stage of the disease, three developed mild clinical symptoms with general enlargement of the lymph nodes, coming on after 8, 17 and 19 days respectively. There was also a slight monocytosis. These changes were reproduced in fresh monkeys by inoculation of excised lymph nodes; 2, 3 and 5 passages were made successfully with the three different strains. Histological examination of the nodes revealed the typical irregular pleomorphic and large-celled hyperplasia seen in man. Of six human volunteers inoculated with heparinized blood or plasma from patients in the acute stage, five remained well. The sixth, who had received 250 ml. of blood, developed typical glandular fever 18 days later.

These results are by no means conclusive. It may be that, as Schultz (1930) and Høring (1933) think, glandular fever is a name covering two or three different diseases. One of these, probably the commonest type, may be caused by a virus; another, probably much less common, may follow infection with *Erysipelothrix monocytogenes*. Further investigations, however, are required to establish this thesis.

Laboratory diagnosis is made by cytological examination of the blood and by the Paul-Bunnell test. Though there is usually a relative lymphocytosis, it is not the absolute nor the relative numbers of the lymphocytes that is characteristic so much as the special morphology of the cells. Blood changes may be present even on the first day of fever, but they do not usually reach their maximum till the end of the second week. They persist for weeks or months. The ability of the blood of glandular fever patients to agglutinate sheep red corpuscles was described by Paul and Bunnell (1932). Normal agglutinins, rarely exceeding a titre of 1/56, may be found in healthy persons (see Smeall 1942), and the heterophile antibody content may rise after injection of horse serum. Observations by Stuart and his colleagues (1934), Bailey and Raffel (1935), Davidsohn (1937), Kristensen (1938), Barrett (1941), and Kilham and Steigman (1942) showed that the normal heterophile antibody can be absorbed completely from the serum by guinea-pig kidney, that that met with in serum disease can be absorbed by guinea-pig kidney and ox red blood corpuscles, and that the heterophile antibody of glandular fever can be absorbed by ox corpuscles but not by guinea-pig kidney. The Paul-Bunnell test, particularly when confirmed by absorption, is of considerable value in diagnosis. A titre of 1/80 or over is highly suggestive of infection, though some workers, such as Halcrow, Owen and Rodger (1943), would put it as low as 1/32. The agglutinin content usually reaches its maximum in 2 to 3 weeks and then falls till after 3 months the titre has often sunk to below diagnostic level. Not all cases, however, give a positive reaction. Shafar and Weir (1913), for example,

Pathogenicity for Man. Veterinarians and others having to do with the care of horses are the most liable to contact glanders. Freshly isolated cultures are highly virulent, and a number of fatal infections have occurred among laboratory workers. The acute form of the malady is the more common in man, most cases terminating fatally within two or three weeks, sometimes within a few days of their inception. As in the horse, the mucous membrane of the nostrils, although not invariably affected, is a place of predilection for the glanders nodules and ulcers. Occasionally the chronic form may appear and linger for months or even years, with spreading ulceration and other features closely resembling those observed in the horse. Recovery from chronic glanders may take place, or the disease may pass into the acute stage.⁴

Path of Entrance. The avenue by which the glanders bacillus usually enters the body of the horse has not been clearly determined. The intact skin probably rarely, if ever, permits entrance, but a slight wound or injury offers a ready portal, as attested by experimentation. The mucous membrane of the nose, especially if slightly abraded, may become the portal of entry, as may the intact conjunctiva, which can be infected by contact with infectious material in two to four hours, sometimes in thirty minutes. Infection by inhalation must be rare, to judge from animal experiments, if, indeed, it ever occurs. According to Nocard, who made a special study of the mode of infection, penetration takes place by way of the alimentary tract in the great majority of cases. There is weighty experimental and other evidence in support of this view.

In man the alimentary tract is certainly not the ordinary channel of entrance; meat from glandered animals has been ingested without resulting infection. Inhalation likewise hardly enters into consideration. Probably infection through a scratch or other break in the skin is the usual origin of human cases.

Diagnosis. In prebacteriological days chronic glanders in the horse was frequently separated from other diseases only with difficulty and a considerable measure of uncertainty. At present the diagnosis of glanders is greatly facilitated by (1) guinea pig inoculation, (2) the mallein test—(a) subcutaneous, (b) ophthalmic, (3) agglutination method, (4) the complement-fixation test.

1. *Guinea Pig Inoculation.* A male guinea pig is injected intraperitoneally with fragments of diseased tissue, scrapings from ulcers, or some of the nasal discharge from a suspected animal. A positive reaction is shown by the testicles becoming red and swollen, usually on the second or third day—the *Straus reaction*. Together with the orchitis (inflammation of the parenchyma of the testicle) there are severe general symptoms which usually culminate in twelve to fifteen days. Grayish nodules are often found in the spleen and other internal organs. The test is not absolutely specific, for Kutscher and Nocard have shown that an analogous orchitis may be produced by other organisms besides the glanders bacillus. It is often, however, of value, especially when, for one reason or another, other tests are inapplicable.

2. *The Mallein Test.* Mallein is the concentrated glycerol broth in which the glanders bacillus has grown, it is prepared in the same manner as tuber-

⁴ *Glanders in man is reported only occasionally; see Panja and Chatterjee Indian Med Gaz., 1943, 78-150, Howe and Miller: Ann. Int. Med., 1947, 26 93.*

give rise to an irregularly remittent type of fever, sometimes accompanied by severe anæmia. The incubation period of the disease is usually about 20 days. In fatal cases death occurs as a rule in 3 to 4 weeks. In patients that recover, convalescence is established after about 5 or 6 weeks, but is often succeeded in a month's time by an eruption of verruga. Second attacks of Oroya fever appear to be uncommon.

Verruga peruana is a disease characterized by the appearance on any part of the body surface of vivid red wart-like eruptions. The disease follows a short time after an attack of Oroya fever, and lasts as a rule for 4 to 6 months. Second attacks may occur, and sometimes a latent infection persists after the subsidence of the skin lesions. The case fatality is very low. There is generally a moderate degree of anæmia, but the parasites in the blood are too few to be seen microscopically, though they can often be revealed by culture. *Bartonella* can be readily cultivated from the local lesions, and can be seen microscopically in stained sections of the excised tissues. Pure cultures, or juice from the nodules, inoculated intradermally above the eyebrow of monkeys give rise after an incubation period of 9-20 days to a local verruga papule. Experimental infection is followed by immunity. In the monkey the spleen appears to play no part in the defence mechanism of the host, such as it does in the rat infected with *Hæmobartonella muris*.

Both Oroya fever and verruga appear to be carried by sandflies, such as *Phlebotomus noguchi* and *Phlebotomus verrucarum* (Shannon 1929, Hertig 1937). In endemic areas latent infections in man appear to be not uncommon (Weinman and Pinkerton 1937). The blood of patients suffering from the disease may contain agglutinins to *Bartonella bacilliformis* in a titre of 1/10 to 1/80 (Howe 1942). Organic arsenic compounds have no curative effect, but chloramphenicol is said to be of value (For further description see Noguchi 1926, Kikuth 1931, 1934, Pittaluga 1938, and a comprehensive review by Weinman 1944)

Infectious Anæmia of rats, caused by *Hæmobartonella muris*, is a disease that is precipitated by splenectomy. Except for certain breeds, such as the Wistar strain, a large proportion of adult rats, both wild and tame, appear to suffer from a latent infection with this organism. In such animals removal of the spleen is followed, usually in 4 or 5 days, by general illness, emaciation, and a severe progressive anæmia. Frequently hæmoglobinuria develops and the animal dies, generally within 14 days. Recovery may, however, occur, but it is liable to be followed by relapses at irregular intervals. Examination of the blood during the acute stage of the disease reveals a high proportion of red cells infected with *Hæmobartonella* (Fig. 216, p 1028). The organisms have been cultivated successfully on leptospiral and other media. In animals that recover they disappear from the blood in 1-5 weeks. Splenectomy in young rats that have not yet become infected with *Hæmobartonella* is without effect, but if a pure culture or infected blood is injected into a splenectomized animal, then typical anæmia develops. Inoculation of a non-infected non-splenectomized animal produces only a mild anæmia. The disease can also be reproduced in young rabbits, guinea-pigs, and white mice by inoculation.

The part played by the spleen is decisive, though in what way it acts is still a matter of conjecture. A quarter of the spleen left *in situ* is sufficient to protect the animal against the disease (Perla and Marmorston-Gottesman 1930). Perla and Marmorston-Gottesman (1932) prepared an aqueous lipid extract of the spleen that neutralized the effect of splenectomy. The same workers found that when

of the benign nature of the winter vomiting disease and the high case fatality of the Jamaican sickness, this seems improbable. Zahorsky first experienced an epidemic at St. Louis in 1904. He observed it during many subsequent winters. The last outbreak he describes was at St. Louis in 1940, when fully 3,000 children were attacked during the first three weeks of February. A similar outbreak at Charleston, S.C., reported by Waring (1942), affected several thousand persons during February and March 1911. All ages are attacked, but infants and young children seem to be specially susceptible. The incubation period is thought to be 2 to 7 days. The disease has an abrupt nocturnal onset, but it may be preceded by abdominal pain or nausea for a day or two. The vomiting is violent, forcible, and often projectile. It lasts for a day or two, and may lead to a state of collapse. Soon after its commencement diarrhoea, accompanied sometimes by colicky pains, sets in. It is seldom profuse, though it may be so. The stools are thin, pale in colour, and very offensive. A temperature of 99°–100° F. is not uncommon. Children sometimes appear profoundly ill. They remain, without food, in a listless or semi-comatose state for 2 or 3 days. Death, however, is rare. A mild inflammation of the upper respiratory tract is often present. The leucocyte count tends to be raised. The clinical diagnosis is made on the presence of an epidemic, persistent vomiting, offensive light-coloured stools, the relatively afebrile course, and the absence of an acute respiratory infection or otitis media. The cause of the disease is unknown. Our own observations, like those of others, have failed to reveal the presence of any known pathogenic organism. The practical absence of cells from the stools suggests that the disease is not primarily an enteritis, nor do epidemiological studies point to a food-borne infection. It seems more probable that infection occurs through the respiratory tract. Zahorsky (1940) distinguishes it from influenzal gastritis, since it may supervene explosively on an outbreak of this disease.

INFECTIOUS CATARRH OF MICE

Nelson (1937) described a disease of mice characterized by rhinitis, otitis media, and a terminal lobar pneumonia. Infection spreads readily by contact, progresses slowly, but ultimately proves fatal. No nasal discharge is visible externally, but a copious semi-fluid white exudate can be withdrawn from the nostrils by a capillary pipette. The disease can be readily transmitted to normal mice by instillation of exudate from the nose, ear or lung. Microscopical examination of nasal or ear discharge reveals the presence of small Gram-negative coccobacilli-form bodies, 0.3–0.4 μ in diameter, arranged singly, in pairs, or small groups, and predominantly extracellular. The organisms cannot be cultivated on ordinary media, but can be grown in tissue culture. Nasal instillation of pure cultures reproduces the disease in mice. The coccobacilli-form bodies are similar to those found by Nelson (1936) in fowl coryza (see below). What group of organisms the coccobacilli-form bodies in these two diseases belong to is doubtful. There is some evidence that they are related to the group of pleuropneumonia-like organisms (see Chapter 40), but further work will be required before this can be asserted with confidence (see Edward 1947). Whether infectious catarrh of mice is allied to the so-called mouse influenza described by Kairies and Schwartz (1936) in Germany is not known.

Austria. The serum of normal horses agglutinates in dilutions of from 1:200 to 1:300, and the reaction is specific only when rather high dilutions (1:500 to 1:3200) are used. The serum from sound animals, however, sometimes agglutinates the glanders bacillus in a dilution as high as 1:500. Occasionally the reaction fails to appear in the serum of glandered animals. The test is liable to the usual difficulties and source of error in the hands of an unskilled observer.

4. *The complement-fixation test* is very accurate but demands special laboratory facilities and is less easy to apply in a practical way than the ophthalmic test.

Immunity and Prophylaxis. Permanent immunity to glanders can neither be conferred by an attack of the disease nor produced by an artificial means. Nocard fed with infectious matter three horses which had previously recovered from the disease, and found that these animals showed no resistance superior to that of a healthy control animal. Similarly, Lobel, Schaaf and Roza⁵ were unable to produce an immunity in guinea pigs or horses by the inoculation of bile-attenuated avirulent bacilli. Chronic glanders may exist for years, and is in no wise a warranty against the sudden development of an acute attack.

No very potent nor characteristic toxic substance has been obtained from cultures of the glanders bacillus, and attempts at immunization with the products of this organism have been eminently unsuccessful. It is stated by a number of observers that repeated injections of mallein will exercise a curative action upon certain forms of recent infection, but experimentally mallein is without immunizing power. The sera of animals treated with mallein injections and the sera of naturally immune animals, such as cattle, are, according to most observers, totally devoid of any preventive or curative value. The most that has been accomplished in the way of immunization is a very moderate augmentation of resistance in dogs injected with small non-fatal doses of living cultures.

The experience of Great Britain shows that the disease may be practically eradicated by slaughtering every animal showing clinical signs of glanders or giving a positive mallein test, and properly disposing of the carcass. By this means the number of horses affected was cut down from 2012 in 1906 to 2 in 1925.

MALLEOMYCES WHITMORI (MALLEOMYCES PSEUDOMALLEI)

Meloidosis, a disease of rodents somewhat similar to glanders, is caused by *Malleomyces whitmori*, a microorganism which closely resembles the glanders bacillus. It has been observed in Rangoon, where it is thought to be primarily a disease of the wild rat and is occasionally communicable to man. *M. whitmori* differs from *M. mallei* in that it is actively motile, liquefies gelatin and attacks carbohydrates more energetically. It grows considerably more rapidly and its colonies on glycerol agar develop a wrinkled, corrugated surface and are quite different in appearance from those of *M. mallei*. A second colony type may be produced, however, which is very similar to the

⁵ Lobel, Schaaf and Roza. *Nederland. Indische Blad Diergeneesk.*, 1941, 53-100

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colonies of the glanders bacillus.⁶ *M. whitmori* is thought by some to be closely related to *Pseudomonas pyocyanea* in many respects and strains have been reported⁷ which produce pyocyanin.

The characteristic lesion of the disease is a small caseous nodule which is found in man in almost any part of the body except the brain. The nodules may coalesce to form large areas of caseation in some cases and in others break down into abscesses. There is some reason to believe that melioidosis in man is often traumatic in origin, either the bacilli entering the injured tissues or the defense mechanism being broken down in part by the injury.⁸ Transmission of the infection among guinea pigs by biting insects, mosquitos and fleas, has been reported.⁹ Rodents usually die within a short time from septicemia, and the lesions appear as small nodules superficially resembling tubercles. Guinea pigs and rabbits are highly susceptible to inoculation. *M. whitmori* also produces the Straus reaction.

⁶ See Finlayson. South African Med. Jour., 1944, 18:113.

⁷ Blanc, Delage and Martin. Ann. Inst. Pasteur., 1943, 69:65.

⁸ Cf. Le Moine, Hasle and Nguyen Duc-Khoi. Bull. Soc. Med. Chirurg. Indochine, 1937, 15:662. Toullec and Huard. *ibid.*, 1937, 15:667, Sudibyo. Geneesk. Tijdschr. v. Nederl.-Indie, 1938, 78:1424 (English summary).

⁹ Blanc and Baltazard. Ann. Inst. Pasteur, 1942, 68:281.

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CORYNEBACTERIUM (THE DIPHTHERIA BACILLUS)¹

As a clinical entity diphtheria dates from the observations of Bretonneau in 1826. The diphtheria bacillus was observed and described by Klebs in 1883, but its etiological relation to the disease was suggested by the investigations of Löffler the following year. Löffler isolated the bacillus observed by Klebs in pure culture from a number of cases of diphtheria but expressly disclaimed the assumption

he found it

find it in all cases of what were apparently clinical diphtheria. The significance of Löffler's findings is now clear, however, for it is known that other bacteria, such as streptococci, can produce a condition in the throat closely resembling diphtheria and that the diphtheria bacillus is not infrequently present in the throat of healthy carriers. Further investigations by other workers indicated that the Klebs-Löffler bacillus was always present in the typical false membrane of diphtheria. In 1888 Roux and Yersin showed that this bacillus formed a soluble toxin which reproduced the characteristic symptoms and lesions of diphtheria and thus demonstrated its etiological relation to the disease.

Morphology and Staining. The diphtheria bacillus is a slender rod ranging from 1 to 6 μ in length and 0.3 to 0.8 μ in breadth. The bacilli are highly pleomorphic, for, in addition to the straight or slightly curved rods, club-shaped and branching forms are not infrequently observed. The presence of the latter, which are a consequence of true branching, is indicative of the close relation of the diphtheria bacillus to some of the higher fungi, and it is classified with the actinomycetes rather than under the Eubacteriales or "true bacteria." Upon completion of cell division a movement designated as snapping occurs, and the bacilli may remain attached but at sharp angles to one another.

The diphtheria bacillus exhibits a marked tendency to stain irregularly. Some cells stain solidly, others take the stain more deeply in transverse bands to give a barred appearance, and in still others deeply staining metachromatic or Babes-Ernst granules are found. A single cell may contain from one to generally not more than five or six such metachromatic granules, they may be found at one or both ends of the cells, particularly those with swollen ends, and when more than two are present the remainder are scattered within the

¹ Diphtheria is considered in detail by Andrewes *et al.*, *Diphtheria. Its Bacteriology, Pathology and Immunology*, Medical Research Council, London, 1922 and by Forbes, *Diphtheria, Past and Present*; mention, John Bale, Sons & Danielsson, London, 1923. Summary of Recent Literature, *Bull. Hyg.*, 1943, 12:969.

of other spirochaetes have been described associated with relapsing fever in other countries; and, as these exhibit certain differences, particularly in their antigenic structure, their virulence to various laboratory animals and, perhaps most important of all, their specificity for their intermediate host—the louse or the tick—they have been regarded by some workers as separate species and named accordingly. Thus we have *Trep. duttoni* of Central Africa, *Trep. novyi* of North America, *Trep. kochi* of East Africa, *Trep. carteri* of India, *Trep. persicum* of Iran, *Trep. usbekistanicum* of Russian Turkestan, *Trep. sogdianum* of Palestine, *Trep. hispanicum* of Spain, *Trep. venezuelense* of South America, and a few more. The close association of strain-peculiarities with a particular species of insect is also proposed as a basis of classification in terms of tick-host (see Davis 1942); the association, however, is not absolute, because cross-infection of various tick species can occur (Johnstone 1942). Other workers, however, regard all these organisms as varieties of the same species.

As seen by dark-ground illumination in the blood of patients during a febrile paroxysm, they are actively motile spiral organisms, with a series of five to ten fairly regular but loose primary waves; during rest their axis is generally straight, but when in motion they momentarily assume various curved and bizarre forms. Their length is variable, and differs with different species; generally it is 10–20 μ . The spirals are 2–3 μ long, and about 1 μ in amplitude. Division occurs by transverse fission; during this process a constriction appears at the middle, and the two organisms draw apart, leaving a thin thread-like connection between them. After separation has occurred, this remnant of the periplast may often be seen attached to one end; by many workers it has been described as a flagellum. Under suitable conditions the organisms are extremely motile, darting rapidly across the field; but in ordinary wet blood films their motion is slower; they move backwards and forwards over a distance of not more than two or three times their own length (Novy and Knapp 1906). Rotation occurs around the long axis. The numbers present in a blood film vary from case to case; at the height of the first pyrexial attack they are often numerous—several organisms to a field—but they may be relatively few and difficult to find. During the decline of the fever their numbers diminish, the organisms become less motile, and not infrequently they assume irregular shapes or accumulate in rosettes (see Fig. 218, p. 1038); these changes are regarded as indicative of lysis or agglutination due to the action of antibodies developing in the host. After the subsidence of the fever they can no longer be found microscopically in the blood; there is evidence, however, to suggest that a few organisms may persist in the blood, since inoculation of blood into animals during the apyrexial interval may give rise to infection. At the onset of the relapse they again become demonstrable microscopically in the blood, though not always in such large numbers as in the first attack. During the interval between the pyrexial attacks the organisms remain latent in the tissues. Experiments with mice and rats suggest that the brain is one of the organs in which infection frequently persists (Heronimus 1928, Schuhardt and Hemphill 1946).

The spirochaetes are best demonstrated by dark-ground illumination; but they may be stained by methylene blue, or preferably by Leishman or Giemsa, with both of which they take on a bluish colour. If the blood films are made in the usual way and allowed to dry in air, the organisms undergo gross distortion and present irregular and coiled forms; if their natural form is to be preserved, they should be wet-fixed.

cell substance. This irregular staining is apparent with Löffler's alkaline methylene blue or with toluidine blue, Neisser's stain² is regarded by many workers as producing an even greater contrast between the heavily and lightly staining portions of the cell substance. Morton and Francisco³ have shown that the metachromatic granules are more readily differentiated when the basic dye is made up in an acid solution.

In stained smears the appearance of diphtheria bacilli is highly characteristic. They may not be identified on morphological grounds alone, however, for many of the pseudodiphtheria bacilli or diphtheroids stain in the same irregular fashion and are similarly pleomorphic. It was formerly thought that there was an association between morphological type and virulence. At present, however, little emphasis is placed upon morphology in this connection, though in a

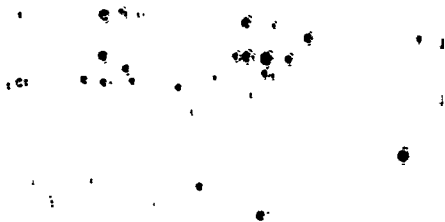


Fig. 138. Colonies of *Corynebacterium diphtheriae* on blood agar. Note the smooth, raised translucent appearance and relatively small size $\times 2$

general way granular types seem to predominate in clinical diphtheria and there appears to be a rough association between morphological type and the

Surface colonies on Löffler's serum medium or on agar are small and gray, when viewed under low magnification they are found to be coarsely granular and somewhat irregular in outline, with ragged or fringed edges. On differential media containing potassium tellurite, colonies of the diphtheria bacillus are dark gray or black because of reduction of the tellurite and readily differentiated from those of contaminating bacteria. Tellurite reduction apparently occurs within the bacterial cell.⁴ It may be noted that although the characteristic morphology of the diphtheria bacillus is apparent in stained smears from colonies on Löffler's medium, smears from colonies on tellurite media are often not characteristic.

* The bacilli are stained with an acid methylene blue solution followed by Bismarck brown.

² Morton and Francisco. Stain Technol., 1942, 17: 27.

⁴ Morton and Anderson. Proc. Soc. Exp. Biol. Med., 1941, 46: 272.

and even to form small tangles. This was even more evident when blood from an immune animal was inoculated into an infected rat; half an hour later the spirochaetes were accumulated in tangled masses of 10 to 20 members, and showed end-to-end agglutination; 1 hour later they were agglutinated into perfect radiating rosettes, and 2 hours later they were very scarce and were mostly immobile. Blood serum from a rat, which had been hyperimmunized by a course of 26 injections of infective blood, had strong immobilizing and agglutinative properties, even when diluted to 1/100. In rats that had recovered naturally from infection Pfeiffer's phenomenon could be produced *in vivo*. Intraperitoneal injection of infective blood into such animals was followed by agglutination and granular degeneration of the spirochaetes; in 10 minutes no free spirochaetes could be found. The altered organisms were rapidly ingested by phagocytes. In hyperimmunized rats the spirochaetes completely disappeared from the peritoneal cavity in 2 minutes. In passively immunized rats the spirochaetes were agglutinated into rosettes, but later these broke up, and free organisms once more became numerous; the animals, however, did not contract infection. The course of antibody production in human beings seems to resemble that in experimental animals (see Cunningham and Fraser 1935).

From these and other experiments, it would appear that during the course of the natural disease immune bodies—chiefly agglutinins, spirochaeticidins and lysins—are developed which are sufficiently powerful to overcome the blood infection and lead to the disappearance of the spirochaetes from the circulation. These organisms remain latent in the brain and other tissues, and when the circulating antibodies have decreased, they once more enter the blood and give rise to a relapse. This stimulates the production of fresh antibodies, which again lead to the disappearance of spirochaetes from the blood. After one or more relapses the active immunity developed by the host is sufficient to prevent further invasion of the blood by the spirochaetes, and an apparent cure results. Whether a true cure results, in the sense that the body is completely rid of spirochaetes, is doubtful. Animal experiments suggest rather that, even though the organisms give no token of their presence, they may yet remain alive in the tissues for weeks or months. Immunity in relapsing fever appears to be an infection-immunity; this corresponds to a state of the host in which, together with a humoral immunity, there is a latent infection of the tissues, which is capable under certain conditions of breaking down the existing immunity (Heronimus 1928). It is a state in which a working equilibrium is established between host and parasite, and like other equilibria is liable to disturbance (see Chapter 51).

There is a considerable amount of evidence to show that after the first attack the spirochaetes in the tissues undergo an antigenic change which renders them insusceptible to the antibodies produced by the host against the original strain. This enables them to invade the blood a second time and give rise to a relapse. The production of antibodies to the so-called "relapse" or "serum-fast" strain is followed by the disappearance of the organisms from the blood once more. A further antigenic change may occur in the tissues enabling the organisms to invade the blood for a third time. As Schuhardt (1912) points out, *in vitro* serological proofs of antigenic variation are more reliable than those depending on cross-resistance tests *in vivo*, unless precautions are taken to prevent relapses, and therefore further possible variation in the immunized test animal. Up to 9 distinguishable antigenic phases have been reported in a single tick-borne strain (Cunningham *et al.* 1934). Tick-borne strains appear to have greater potentialities for variation than louse-borne strains. Successive variation with relapses occurs after the inoculation of a single spirochaete (Schuhardt and Wilkerson 1951). Relapses in

Physiology. The optimum growth temperature for the diphtheria bacillus is 34° to 36° C. and it grows well at 37° C.; growth will take place over the range from 15° to 40° C. An alkaline reaction is required, pH 7.8 to 8.0, and free access to air is essential, for growth under anaerobic conditions is sparse.⁵

In primary isolation the diphtheria bacillus is best cultivated on enriched media. Growth is rapid on Löffler's serum medium (3 parts of beef or sheep serum and 1 part of 1 per cent dextrose broth coagulated in slant form by inspissation) and minute but visible colonies appear after twelve to twenty-four hours' incubation. In recent years a variety of differential and selective media have been introduced, all of which contain potassium tellurite. The better known of these are the chocolate agar-tellurite medium of Anderson and his co-workers,⁶ which has been subject to minor modifications by a number of other workers, such as Neill's medium and Hoyle's medium which are



Fig. 139. The diphtheria bacillus, *gravis* strain, pure culture on blood agar. Methylene blue stain. Note the bipolar staining and the club-shaped forms. The lightly stained cells with deeply stained areas are characteristic of *gravis* morphology. $\times 1200$.

used in England, and the various media developed by Clauberg, whose inspissated serum-glycerol-tellurite medium has been widely tested. There is general agreement that the proportion of positive cultures is somewhat higher with the Clauberg medium than with Löffler's medium; whether the heated blood-tellurite media are superior to Löffler's medium is not clear. As indicated above, the characteristic morphology of the diphtheria bacillus is not always seen in smears from colonies on tellurite media and, therefore, in some laboratories both Löffler's medium and a differential tellurite medium are inoculated and the former used for microscopic examination if typical colonies appear on the differential medium.

Enriched media are not, however, essential to the growth of the diphtheria bacillus, for this microorganism can be cultivated on ordinary nutrient and in-

⁵ Strains of virulent diphtheria bacilli which grow more luxuriantly under anaerobic conditions than in the presence of air have, however, been reported Cf. Emilio Giorn. di Batteriol. e Immunol., 1938, 21:256.

⁶ Anderson, Happold, McLeod and Thomson. Jour. Path. Bact., 1931, 34:667.

1-2 ml. of fresh whole blood, or of clot ground up in saline, should be injected intraperitoneally into a susceptible animal, either the mouse or the young white rat. Spirochætes may not become visible microscopically in the mouse's blood for 2 to 3 days, and sometimes for much longer. A drop or two of blood from the tail should be examined every day for at least a fortnight from the second day onwards, if the animal does not die of the infection.

Spirochætes may sometimes be demonstrated by animal inoculation in the blood of patients for weeks after the primary attack. According to Chung (1938) the urine and prostatic fluid are sometimes infective. In making rodent surveys, the spleen, heart, or brain tissue should be ground up in saline and inoculated subcutaneously into white mice; the blood is then examined daily for spirochætes in the usual way. It is worth noting that the Wassermann reaction may be positive in relapsing fever; and that, in louse-borne infection, the Weil-Felix test may be positive for *Proteus* OX K (Robinson 1942, Zarafonitis *et al.* 1946). A serological method of diagnosis, using spirochætal suspensions made from saponin-lysed blood of heavily infected animals, is described by Stein (1944), and a complement-fixation test, carried out with an antigen prepared from the allantoic fluid of infected chick embryos, by Wolstenholme and Gear (1948).

Prophylaxis and Treatment.

Little can be done by the individual to protect himself against infection in an endemic area beyond avoiding lice or ticks. Inoculation of living cultures, or sometimes of cultures killed by heating to 60° C. for 30 minutes, is said to give rise to the production of lysins. Persons so vaccinated may apparently resist infection with small doses of living spirochætes (Aristowsky and Wainstein 1929*a, b*). Like most spirochætal diseases, relapsing fever is readily cut short by injection of salvarsan or neoarsphenamine. A single suitable dose often suffices to cure the disease and prevent relapses (Wenyon 1926). However, just as "serum-fast" variants may develop during the course of the disease, so "arsenic fast" strains may be encountered, which are little affected by salvarsan. Infections with such strains may be treated by sodium potassium bismuth tartrate (Todd 1930). The serum of convalescent patients may be used therapeutically, but the little experimental and clinical information available about this method of treatment is not encouraging (Adler and Ashbel 1937, Wolman 1944).

The experimental disease in rodents can be cured by penicillin in large doses (see Lourie and Collier 1943); but spirochætes latent in the brain appear to be inaccessible to the drug (Schuhardt and O'Bryan 1944, 1945). Effective treatment of the disease in man with penicillin is reported by Taft and Pike (1945) and Ingraham and Lapenta (1946), and with aureomycin by Yeo (1950).

AVIAN SPIROCHÆTOSIS

In 1891 Sakharoff described a disease of geese that appeared every year in certain stations on the Transcaucasian railway, and resulted in a high mortality—80 per cent. Examination of the blood revealed the presence of spirochætes closely resembling those of human relapsing fever. Clinically, the infected goose went off its feed, remained apathetic in a sitting-down posture, and died of exhaustion after a week or more; sometimes it developed diarrhoea, and its joints became affected. *Post mortem*, there was fatty degeneration of the heart and

fusion media. Growth is somewhat scanty on the former but good on the fresh meat infusions. The intensive investigations of Mueller⁷ have shed considerable light on the growth requirements of these bacilli. Some strains, including the well-known Park 8, may be cultivated on synthetic solutions containing a number of amino acids together with small quantities of nicotinic acid, β -alanine or pantothenic acid, and pimelic acid. It has been suggested that pimelic acid is utilized by this bacterium for the synthesis of biotin since growth is stimulated by biotin in the absence of pimelic acid. Recently isolated strains also require oleic acid for development, especially if the inoculum is small. Nutritive requirements differ somewhat from one strain to another and a general statement is not possible.

The diphtheria bacillus does not liquefy gelatin or digest coagulated protein. Indol is not formed⁸ and nitrates are reduced to nitrites. All strains form acid

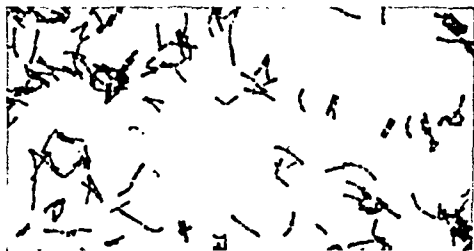


Fig. 140. The diphtheria bacillus, *intermedium* strain, pure culture on blood agar. Methylene blue stain. Note the irregular staining and barred appearance characteristic of the *intermedium* variety. $\times 1200$.

but no gas from dextrose and levulose, and some strains ferment dextrin, glycogen, starch, galactose, maltose and glycerol. There appear to be no well defined biochemical groups among these bacilli. The fermentation of dextrose is of some interest in that propionic acid is formed. Other products of the fermentation include lactic, acetic, formic and succinic acids and ethyl alcohol.

In ordinary culture media the diphtheria bacillus may retain its vitality for relatively long periods of time. It will live six to eight weeks on agar, five to six months on blood serum, twelve to fifteen months on dextrose blood serum, and as long as three months in particles of diphtheric membrane. Although virulence is ordinarily reduced by continued culture on laboratory media, some strains remain fully virulent, i.e., toxigenic, on prolonged cultivation. Löffler recorded one instance in which virulence was maintained over 77

⁷ Summarized by Mueller: Jour. Bact., 1938, 36:499, Bact. Rev., 1940, 4:97.

⁸ The test with sulfuric acid and potassium nitrate may be positive because of the formation of indol and acetic acid, but no color is produced with Ehrlich's reagent, *p*-dimethyl amido benzaldehyde (Fischer: Centralbl. f. Bakt., 1921, 57:254).

to be responsible; in the old world *Argas persicus* is the tick that has been chiefly incriminated. Marchoux and Salimbeni (1903) found that ticks might remain infective for 5 months after biting a diseased fowl. According to Hindle (1912) ticks may transmit the infection to their progeny, and these again to the next generation, without having had an infective feed in the meantime.

The disease occurs in ducks and in turkeys (Hoffman and Jackson 1946, McNeil *et al.* 1949) as well as in fowls and geese. It is probable that the causative organism is the same in each species; its proper name therefore is *Treponema anserinum* (Wenyon 1926). Those requiring further information on avian spirochaetosis are referred to the monograph of Knowles, Gupta, and Basu (1932) who, besides giving a bibliographical review of the subject, have made a number of observations themselves, particularly on the mechanism by which immunity develops.

Both penicillin and the organic arsenicals are reported to be therapeutically effective (see McNeil *et al.* 1949).

Blood Spirochaetoses in other Animals.

Cattle suffer from a spirochaetosis caused by *Treponema theileri*; infection is conveyed, at least in South Africa, by the tick *Margaropus decoloratus*. A similar disease in horses and in sheep, probably due to the same organism, *Trep. theileri*, has also been reported by Theiler in South Africa. Spirochaetes of the relapsing fever type have been observed in the blood of elephants, camels, antelopes, monkeys, and some other mammals (Wenyon 1926).

VINCENT'S ANGINA AND CERTAIN RELATED INFECTIONS

There are certain necrotic and gangrenous infective processes in human beings, such as ulcero-membranous gingivitis, hospital gangrene, noma, foetid bronchitis, and gangrenous laryngitis, in which spirochaetes have frequently been demonstrated. Of these, one of the chief is the so-called spirillum described by Vincent (1896, 1899), now known as *Treponema vincenti* (see Chapter 38). It is not clear whether Vincent's spirillum is responsible for the necrotic lesions in which it is found, or whether it is a mere secondary invader. The fact that it is often present in scrapings of the gingivo-dental fold in apparently healthy mouths has led many observers to doubt its aetiological rôle in the inflammatory diseases just mentioned. Black (1938), for example, found Vincent's spirillum in 60 per cent. of children under 12 years of age, fusiform bacilli in 94 per cent., and both together in 18-63 per cent. according to age. He regards these organisms as members of the normal flora of the mouth, which multiply when conditions are favourable, but which are without pathogenic action. Recent work (see p. 2127) has shown that some of the gingivo-stomatitis of children is a primary herpetic infection; but there is nevertheless a group of diseases characterized by ulceration of the gums and throat, in which no virus has so far been demonstrated, but in which Vincent's spirilla are abundant. The organisms are often found associated with a characteristic fusiform bacillus, likewise described by Vincent (1896, see Chapter 18). It has been suggested (Tunncliffe 1906) that *Trep. vincenti* and the fusiform bacillus represent two phases of the same organism; but the balance of evidence is definitely against this view. Since we know that strict anaerobes are unable to grow in healthy tissue, the finding of large numbers of spirochaetes and fusiform bacilli

transfers covering a period of twenty-seven months. The bacilli are unusually susceptible to heat; a suspension or broth culture is killed by holding at 58° C. for ten minutes. In diphtheric membrane they are considerably more resistant.

Toxin. With the possible exception of the Shiga dysentery bacillus, the diphtheria bacillus is the only aerobic bacterium that produces a powerful exotoxin comparable to those formed by the sporulating anaerobes. Filtrates from broth cultures are not so toxic as those of the tetanus and botulinus bacilli; exceptionally potent filtrates may contain as much as 1000 guinea-pig MLD's per milliliter. It may be noted that in regard to the diphtheria bacillus virulence and toxigenicity are synonymous. The *virulence test*, the inoculation of guinea pigs with broth culture of the bacillus, is, then, a test of the ability of the bacilli to form toxin.

The production of toxin by the diphtheria bacillus is markedly influenced by environmental and nutritive conditions; even strongly toxigenic strains may produce little or no toxin under unfavorable conditions. A slightly alkaline reaction, pH 7.8 to 8.0, is essential, for an acid reaction strongly exhibits toxin formation. Free access to air is also necessary, and for the production of toxin the bacilli are cultivated in thin layers of beef infusion broth. Maximum amounts of toxin are found after seven to ten days' incubation at 36° to 37° C.

An infusion medium, beef infusion, containing adequate amounts of peptone (2 per cent) has long been regarded as essential to the production of maximum amounts of toxin. The marketed brands of peptone are variable in this respect, some being much better for the production of toxin than others. The presence of protein or large peptone molecules is not essential to toxin formation, however, and potent toxins may be produced in chemically defined media containing appropriate amino acids and other compounds. The critical factor is not the quality of peptone or other source of nitrogen, as once thought, but the concentration of iron in the medium. Pappenheimer and Johnson⁹ found that maximum toxin production occurs only over a narrow range of iron concentration, the optimum being 0.14 µg./ml., and 5.0 µg./ml. almost completely inhibits its formation. Calcium, sodium and potassium also affect toxin production but the concentrations are not so critical. Mueller¹⁰ has suggested that the small amount of toxin produced in the presence of large amounts of iron represents normal production while the increased production under conditions of iron starvation is possibly the result of a compensatory mechanism in which the toxin molecule takes part in some process ordinarily catalyzed by an iron-containing enzyme. Pappenheimer has reported¹¹ that four mols of porphyrin and one mol of toxin disappear from the filtrate for every four mols of iron added to the culture medium, suggesting that the toxin is the protein moiety of an iron porphyrin respiratory enzyme of the diphtheria bacillus. However this may be, potent diphtheria toxin is formed in a reproducible semi-synthetic medium containing hydrolyzed casein, nicotinic and pimelic acids, cystine, maltose, calcium and iron that gives better and more consistent toxin production than the complex infusion media.¹²

⁹ Pappenheimer and Johnson. *Brit. Jour. Exp. Path.*, 1936, 17:335.

¹⁰ Mueller *Jour. Immunol.*, 1941, 42:343.

¹¹ Pappenheimer. *Jour. Biol. Chem.*, 1947, 167:251.

¹² Mueller and Miller *Jour. Immunol.*, 1941, 40 21.

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The properties of the diphtheria toxin are similar to those of the other soluble toxins which have been discussed elsewhere (p. 202) and need not be considered at length here. Suffice it to say that the toxin is unstable to slight acidities, i.e., a pH of 6 or less, is heat-labile and apparently protein in nature. The production of toxin in synthetic nutrient solutions containing insignificant amounts of high molecular weight substances allows its separation in a relatively high degree of purity from such solutions. The minimum amount of toxin with diphtheria toxin. The guinea-pig MLD of this material is about 0.001 mg.

Diphtheria toxin is an excellent antigen and gives rise to high titer antitoxic sera. The standardization of diphtheria toxin and antitoxin is discussed elsewhere (p. 288). It has been noted from time to time that various substances, including bile, ascorbic acid (vitamin C), sterols such as lanolin, cholesterol and the like, will neutralize diphtheria toxin. The significance of such observations is not entirely clear. Generally relatively large amounts are required to neutralize small quantities of toxin. One or two attempts have been made to utilize ascorbic acid in the treatment of clinical diphtheria but without significant results.

It may be noted that there is great variation from strain to strain of diphtheria bacilli in the ability to produce toxin, ranging from the highly toxigenic strains to relatively or completely atoxigenic ones. The well known Park No. 8 strain is one of the good toxin producers and has been widely used for this purpose. It has been observed by some workers that pure cultures of toxigenic strains will, at times, give rise to weaker toxin producers or completely atoxigenic varieties.

Variation. As in other groups of bacteria, smooth and rough variants of the diphtheria bacillus have been observed and the type of colony formation has been found to be correlated with morphology and virulence; the S variant is the more virulent and the form commonly found in acute cases of diphtheria. Morphological and biochemical variation observed in the diphtheria bacilli has been critically reviewed by Morton.¹⁴ As a consequence of its pleomorphism and tendency to branching and the like, the diphtheria bacillus has been thought by some to undergo the cyclic transformations of a complex life cycle including the production of gonidial and filterable forms. This concept is not generally accepted. It has also been suggested that some of the diphtheroid bacilli are diphtheria bacilli which have lost their ability to form toxin.

Types. Morphological types of the diphtheria bacillus were described by Anderson and others¹⁵ in England in 1931 and since have been found in various parts of the world. These were of very considerable interest when first observed for there appeared to be an association, especially in England, between the type and the degree of severity in the clinical manifestations of the disease. Those designated as the *gravis* and *intermedius* types were found in severe cases of diphtheria, and the *mitis* type in the milder cases. The associa-

¹⁴ Pappenheimer Jour. Biol. Chem., 1937, 120 453. see also Lange and Brit. Jour. Exp. Path., 1941, 22 255.

¹⁵ Morton Bact. Rev., 1940, 4 177.

¹⁶ See the comprehensive review by McLeod Bact. Rev., 1943, 7 1.

type of syphilis Except in congenital syphilis, in which the disease is generalized from the start, infection is usually rendered evident by the development of a primary lesion or chancre. This appears within a month of infection, and is accompanied by enlargement of the focal lymphatic nodes. From 6 to 12 weeks after the appearance of the primary chancre, the secondary stage of the disease sets in; this is marked by constitutional symptoms, cutaneous lesions, enlargement of the lymph nodes, and often affections of the bones, joints, eyes, and other organs. The secondary passes over insensibly into the tertiary stage, which may persist for years; this is characterized by the development of ulcerating necrotic lesions of the skin and mucous membranes and by gummata of the internal organs. Years after the contraction of the disease disorders of the nervous system may appear, such as tabes dorsalis and general paralysis; these are sometimes referred to as quaternary or parasyphilitic affections.

Up to the commencement of the secondary stage, syphilis is clinically a localized disease; bacteriologically, however, it appears probable that infection becomes generalized soon after infection. Kolle and Evers (1926b) infected rabbits by cutaneous or subcutaneous inoculation into the scrotum with syphilitic material, removed the inguinal nodes after varying periods, and injected these into fresh animals. By this means they found that the nodes were infective within 30 minutes of the scrotal inoculation. Working with guinea-pigs, they were able to show that some of the spirochaetes reached the focal nodes within 5 minutes of cutaneous inoculation of the scrotum. In apes the time elapsing between infection and invasion of the nodes is probably longer; Metchnikoff and Roux (1905), for example, found that chimpanzees anointed locally with calomel ointment, 1 to 2 hours after cutaneous inoculation, never developed syphilis. Taking this experimental evidence in conjunction with the fact that local disinfective measures in human beings are comparatively valueless unless practised within an hour or two after exposure to infection, we may conclude that the spirochaetes rapidly invade the tissues, even though they give no clinical sign of their presence. Invasion by only a few organisms may be sufficient to establish infection. In the rabbit, Magnuson, Eagle and Fleischman (1948) infected 50 per cent. of rabbits receiving as few as four spirochaetes intracutaneously; intratesticularly an average of one spirochaete was regularly infective. During the primary stage of the disease, spirochaetes are found in the local chancre, and can sometimes be demonstrated in the blood. Thus, Uhlenhuth and Mulzer (1913) drew off the blood of patients with primary and secondary syphilis, defibrinated it, and injected it into the testicles and scrotum of rabbits; the whole operation was completed within 10 minutes. Syphilis developed in 67 per cent. of the animals inoculated from patients with primary, and in 70 per cent. of those inoculated from patients with secondary syphilis. Spirochaetes are present in all the secondary lesions, and may be excreted in the semen (Uhlenhuth and Mulzer 1913). In the tertiary lesions such as gummata they are demonstrable, but only in small numbers; their virulence, however, appears to be maintained. Noguchi and Moore (1913) found them in the brain of patients dying of general paralysis, they were seen in all the layers of the cortex with the exception of the outer or neuroglial layer. In congenital syphilis spirochaetes are distributed in large numbers throughout the viscera, particularly the liver, lungs, spleen, and suprarenals. Both in congenital and acquired syphilis the organisms may remain latent for long periods of time without giving rise to any clinical manifestations of disease.

tion was less clear on the Continent, and there appeared to be little or no relation in the United States, where the *mitis* type occurs much more frequently and perhaps only 1 per cent of the strains are of the *gravis* type. The toxins produced by the three types are equally neutralizable by the ordinary antitoxic sera, but the *mitis* strains produce toxin somewhat more actively *in vitro* than the *gravis* and *intermedius* types. The three types also appear to be equally virulent for the guinea pig. By now it is more or less generally agreed that the differentiation of these types is not significantly related to clinical severity, but has been useful from an epidemiological point of view.

These types may be differentiated by their colonial form on tellurite media. The *gravis* type produces irregular striated colonies predominantly gray in color; the *mitis* type, small, round, smooth, convex colonies predominantly



Fig. 141. The varieties of the diphtheria bacillus on chocolate-tellurite agar. Left, *mitis* type; note the characteristic raised, small black colony. Center, *intermedius* type; the lighter color, beginning radial striation and small size are apparent. Right, *gravis* type, the gray color, larger size, raised center and radial striation are evident.

black in color and softer in consistency; and the colonial form of the *intermedius* type lies between these. Colonial differences are also apparent on certain other media, such as trypsin-serum agar and a potato extract-cystine-water blue-glycerol medium devised by Clauberg. On fresh blood agar the *mitis* type is usually hemolytic, the *intermedius* type non-hemolytic, and the *gravis* type usually non-hemolytic. A further distinction is the fermentation of glycogen and starch by the *gravis* type; but other biochemical tests do not differentiate these types.

There is some association between colonial type and the morphology of the bacillary forms. Those of the *gravis* type show one or two deeply staining areas, the remainder of the cell staining very lightly; metachromatic granules are seldom observed. Bacilli of the *mitis* variety stain irregularly and contain very many well-developed metachromatic granules. The *intermedius* forms exhibit the familiar barred appearance. While some 80 per cent of the *intermedius* variety conform to this morphology, only 50 to 60 per cent of the *gravis* strains are typical, the remainder resembling the *mitis* and *inter-*

(Kolle and Schlossberger 1928), and can be transmitted, without producing symptoms, through a series of mice (see Levaditi *et al.* 1948). There is evidence that the organisms actually multiply in the tissues of the mouse; thus for 6 to 8 weeks after infection the organs frequently prove negative, but after that time, and apparently for the remainder of the mouse's life, they are positive. In spite of this development in the tissues, the organisms never give rise to symptoms of disease. It should be noted, however, that the expectation of life of the mouse appears to be decreased by infection (Rosahn 1952).

The co-existence of symptom immunity and spirochaetes in the tissues, capable of producing syphilis when transferred to normal animals, is undoubted. The existence of immunity in a spirochaete-free animal is harder to establish, because only small portions of rabbit tissue, such as a lymph node, can be tested for infective spirochaetes, if the animal is to survive for subsequent tests of immunity; and a negative lymph node is no certain proof of uninfected tissue elsewhere in the body.

Tests with animals apparently cleared of their first infection by chemotherapy suggest that an immunity develops, which may be a partial symptom immunity, or complete in that when spirochaetes are inoculated, they do not survive in the tissues.

Brown and Pearce (1921) treated rabbits with arsphenamine shortly after the development of the primary chancre, and 5 days later reinoculated them with the same strain as was originally used. Nearly all the treated animals developed chancres, whereas the untreated controls did not. This indicates that animals treated in the early stage of the disease can be rendered fully susceptible again. Chesney and Kemp (1925) obtained evidence suggesting that if treatment was begun early the animals almost always became susceptible to a second infection, whereas if it was begun late they generally proved refractory to a second infection. Further experiments (Chesney and Kemp 1926, Chesney, Halley, and Kemp 1927), however, seemed to show that the immunity to reinfection of rabbits treated late in the disease was not as complete as was originally supposed. Kolle and Prigge (1927) treated their rabbits with neosalvarsan in the late stage of the disease—75 to 250 days after infection. Reinfection gave rise to no symptoms, but inoculation experiments showed that the spirochaetes had gained access to the tissues; in non-reinfected controls the tissues proved sterile. This indicates that animals treated in the late stage of the disease, and cured of infection, remain clinically immune; they develop no symptoms of disease on reinfection, but they are not sufficiently resistant to prevent the organisms from gaining access to the tissues. Uhlenhuth and Grossmann (1928) treated their rabbits with neosalvarsan in the late stage of the disease—107 to 506 days after infection. Reinfection with the homologous strain performed 3 to 10 months after treatment gave rise to no symptoms; and in only 4 out of 11 rabbits were spirochaetes demonstrated in the tissues. This indicates that a certain proportion of rabbits treated in the late stage of the disease remain resistant to fresh infection. (See also Breinl 1935.)

Grossmann (1933) contend that this immunity is (1927, 1929, 1934) that it is a symptom immunity. Similar results were obtained by more recent workers, using penicillin to produce a rapid termination of the immunizing infection. A proportion of rabbits so tested developed a complete or a symptom immunity as the result of a 3-weeks' infection; 12-14 weeks' infection conferred maximum immunity to reinoculation (Magnuson and Rosenau 1948, Magnuson *et al.* 1950). The proportion of rabbits with solid immunity was greater after a 10-weeks' than after a 5-weeks' infection (Arnold *et al.* 1950a); and latent infection of 8-months' duration conferred an immunity lasting 6-23 months (Arnold *et al.* 1950b; see also McLeod and Arnold 1951).

In all the foregoing experiments the criterion of complete immunity was usually absence of spirochaetes in excised lymph nodes. In some cases we may note, however, that such

medius forms. Furthermore, 5 to 20 per cent of the *mitis* strains show barred forms.

Serological investigation has shown that while these three types are antigenically distinct from one another, the types are not necessarily homogeneous. The *mitis* strains are heterogeneous, the *gravis* strains fall for the most part into two types, and the *intermedius* strains are relatively homogeneous, some strains showing relationship to the *gravis* types.

Not all strains showing the morphological and biochemical characteristics of these types are virulent, i.e., toxigenic, diphtheria bacilli. Since the diphtheria bacillus is differentiated on the basis of the formation of immunologically specific toxin, it is apparent that *mitis*, *gravis* and *intermedius* types of diphtheroid bacilli occur. In the series studied by Frobisher,¹⁶ for example, only 10 per cent of the strains typed as *gravis* were toxigenic. Furthermore, a certain proportion of toxigenic strains cannot be allocated into one or another

CHARACTERISTICS OF THE DIPHTHERIA BACILLUS TYPES

Type			<i>mitis</i>	<i>intermedius</i>	<i>gravis</i>
Morphology	Microscopic		Usually long, with many metachromatic granules—80 per cent typical	Usually barred, club forms common—80 per cent typical	Short, evenly staining—50-60 per cent typical
	Colonial	Tellurite	Small, round, smooth, convex, black with grayish periphery	Small, flat, dull, gray raised center	Large, irregular dull gray, raised center, radial striations
		Chocolate	Smooth, semi-opaque, glistening	Flat, dry, opaque, slight greenish zone	Flat, dry, matt opaque
		Broth	Uniform turbidity sometimes slightly granular, with pellicle	Finely granular turbidity	Granular, flakes pellicle—variable
	Physiology	Fermentation of	Glycogen	—	—
		Starch	—	—	+
Hemolysis		+	—	—	
Immunity			Heterogeneous	Relatively homogeneous	Two main types

of these types. The proportion of indeterminate strains is said to be higher when diphtheria is mild. Anderson *et al.*⁶ found about 5 per cent of their strains (British) were not typable and 44 per cent of the strains found by Seligmann¹⁷ in New York City in 1940 were indeterminate.

Pathogenicity for Man. Diphtheria is primarily a disease of childhood and the age incidence is an expression of waning passive immunity of maternal origin and the development of an active immunity on the one hand, and the risk of exposure on the other. The very young child is passively protected and not exposed to great risk of infection, but by school age the immunity has disappeared in large part and risk of exposure to infection is tremendously increased with entrance into school. The adolescent and adult have acquired an active immunity as a consequence of clinical or, more commonly, inapparent

¹⁶ Frobisher, Amer. Jour. Pub. Health, 1942, 32:709.

¹⁷ Seligmann, Amer. Jour. Hyg., Sec. B, 1941, 34:125.

The antibody responsible for complement fixation or flocculation with Wassermann antigen (see below) has little relation to immunity. It does not react with intact spirochaetes; and its titre is high in the early stages of infection, when there is little immunity, and may be low or absent in immune animals.

Diagnosis.

In the primary stage of the disease, *Trep. pallidum* may be demonstrated in the chancre.

The superficial part of the lesion should be cleansed by gentle swabbing with saline, and exudate should be drawn from the base of the chancre for examination; this may conveniently be obtained by applying a small suction-cup with the usual rubber-ball attachment. The serous exudate should be examined under dark-ground illumination. The morphology of *Trep. pallidum* is described on p. 1040. The eye-piece micrometer devised by Barnard (1923), with which an approximate measurement of the spirals can be made with ease and rapidity, forms a most useful aid in the identification of this organism. In exudate stored in sealed capillary tubes at 37° C. the spirochaetes are said to remain motile for 1-2 weeks (Lumsden 1947). (For examples of stains for demonstrating the spirochaetes, see Goldsworthy and Ward 1942, Campbell and Rosahn 1950.)

It cannot be too strongly emphasized that the identification of *Trep. pallidum* in a primary chancre requires the expert knowledge which comes only with long experience. It depends on the recognition of fine differences in morphology, without

the assistance of characteristic differences in staining reaction, or of confirmatory tests on pure cultures. It is fortunate that the primary penile chancre provides the large majority of cases which the bacteriologist is called on to examine, since the spirochaetal flora in such lesions is usually not copious, and consists mainly of species, such as *Trep. refringens*, which are readily distinguished from *Trep. pallidum*. Chancres in other situations, such as the lip, or primary genital chancres in the female, are far more likely to show a complex spirochaetal flora, including species closely resembling *Trep. pallidum*; and, in such cases, the greatest caution should be observed in basing a diagnosis of



FIG. 297—*Treponema pallidum*.

In material scraped from a hard chancre. Fontana
($\times 1000$).

syphilis on a microscopical examination alone. It happens that a penile chancre usually leads to a suspicion of syphilis at an earlier stage than a genital chancre in the female, or an extra-genital chancre in either sex; so that the latter lesions tend to be overlooked in the early stages, and, by the time they have attracted attention, the infection has usually reached the stage at which the Wassermann reaction is positive, thus providing a most valuable check on the result of the direct microscopical examination.

infection. Thus clinical diphtheria is most common in the five to fourteen age group though the carrier state and inapparent infection are probably no more so than in the higher age groups. Though diphtheria is in large part preventable by artificial immunization, particularly of the preschool child, it continues to be an important disease and 17,612 cases with 1494 deaths were reported in the United States in 1945.

Diphtheria in man is usually a local infection of the mucous surfaces. The pharynx is most commonly affected, but infection of the larynx, or membranous croup, and nasal diphtheria, or membranous rhinitis, are not infrequently observed. Diphtheritic infections of the conjunctiva and of the middle ear are less common and cutaneous or wound diphtheria is only occasionally observed. The last, however, may assume considerable proportions under certain circumstances. Ulcerative diphtheria of the skin, sometimes called desert sore or tropical ulcer, has been observed in epidemic form in Haifa,¹⁸ and ulcers of the deep, punched out type occurred with some frequency in troops living under combat conditions in the south and central Pacific areas during World War II.¹⁹ Infection of the mucous surfaces of the genital organs is occasionally found. The invasion of other localities is rare; primary infection of the lungs and diphtheritic meningitis have been observed, and infection of the umbilicus in the new-born has been reported.

It has long been known that diphtheria bacilli are not often found in the internal organs, but the frequency with which such infection occurs is not definitely known. Diphtheria bacillus septicemia is occasionally observed,²⁰ however, and it is of interest to note here that a few cases of acute vegetative endocarditis caused by the diphtheria bacillus have been reported.²¹

The symptoms and lesions produced are due partly to the presence of the bacillus and partly to its toxin. The chief local consequence of infection is a degeneration of the epithelial cells, extending to the underlying tissues and accompanied by a profuse fibrinous exudation, and the characteristic diphtheritic membrane, containing fibrin, dead tissue cells, leucocytes and bacteria, is formed on the affected surface. The mechanical interference of the membrane with breathing may assume significant proportions and even necessitate intubation or tracheotomy.

Although diphtheria toxin undoubtedly plays a part in the formation of the membrane, its systemic effects following absorption are by far the most important, and diphtheria is, like tetanus, essentially a toxemia. The organs most severely affected are the kidneys, heart and nerves. A variety of lesions may be found in the kidneys, an acute interstitial nephritis being the most common. The lesions in the heart consist commonly of a fatty degeneration in the muscle fibers, which may be very extensive. Fatty degeneration also occurs both in the myelin sheath of the peripheral nerves and in the white matter of the brain and cord. These changes in muscle and nerve account for the serious cardiac weakness often observed in diphtheria and the frequent occurrence of the more or less extensive paralysis which so commonly follows an attack

¹⁸ Gill. Arch. Dermat. Syph., 1945, 51:243

¹⁹ Liebow, MacLean, Bumstead and Welt Arch. Int. Med., 1946, 78:255.

²⁰ The literature is reviewed by Kaschel: Ztschr. f. Kinderheilk., 1938, 59:437.

²¹ Cf. Buddingh and Anderson. Arch. Int. Med., 1937, 59:597.

observed correlation with clinical findings, for which there is, as yet, no satisfactory explanation. The methods of performing the test are legion (see Reports 1918a, 1924, 1929, 1934, Cumming *et al.* 1935, Vogelsang 1940, Osmond 1946, Price 1950) and many modifications have been suggested to increase its reliability and sensitivity (see Osler and Strauss 1952). The principles are, however, in no way different from those outlined in Chapter 7, in connection with complement fixation in general.

Three reagents are concerned in the reaction—antigen, patient's serum, and complement. When these have been allowed to react for a suitable period—1 hour at 37° C, or overnight in the ice-box—sensitized red cells are added, and the mixtures are incubated for 1 hour at 37° C. to determine the presence or absence of hæmolysis, indicating the presence or absence of unabsorbed complement. To establish a positive reaction it must be shown (a) that the patient's serum alone absorbs no complement, or at most a small fraction of that present in the test mixture; (b) that the same is true of the antigen; and (c) that the mixture of serum and antigen absorbs an amount of complement greatly in excess of the sum of the small amounts absorbed by each of these reagents separately. Thus the essential tubes in carrying out a Wassermann reaction are (1) the antigen control, (2) the serum control, and (3) the test proper. The first control will serve for the whole series of tests carried out on any one day, the second must be put up for each serum tested. It is, however, obviously desirable to obtain a more accurate measure of the strength of the reaction than is possible by such a simple method as this; and most of the methods in actual use are designed to yield a roughly quantitative result, which will enable the reactions to be graded into strongly positive, positive, weakly positive, doubtful, and negative.

There are three variables concerned in the reaction—antigen, serum, and complement; clearly, we may obtain quantitative results by holding any two of these constant, and varying the third. The antigen is usually kept constant, and the complement or serum varied. A commonly employed method of complement titration is to allow the serum-antigen mixtures to react with 3 and 5, or with 2, 4, 6 and 8 M.H.D. of complement. The serum and antigen controls are put up with the smallest amount of complement employed in the test. In this way we grade the strength of a reacting serum in terms of the amount of complement which is fixed in the presence of a constant amount of serum and antigen.

An alternative method, advocated by several workers, is to hold antigen and complement constant, and vary the amount of patient's serum, testing it in progressive dilutions of, say, 1/2.5, 1/5, 1/10, 1/20, 1/40, though it is not usual to employ more than three dilutions. This method has the advantage of giving a direct measure of the concentration of the Wassermann-antibody in the serum under test. The serum control is, of course, put up with the largest amount of serum employed in the actual test.

In both cases it is necessary first to determine the minimal hæmolytic dose of complement. For example, duplicate titration series of fresh guinea-pig serum are set up, one with added antigen, the other with saline. After one hour at 37° C., red cells sensitized with at least 5 M.H.D. of a hæmolytic serum are added to each of the tubes, which are held for one hour at 37° C. The volume of liquid in each tube is adjusted at all stages to correspond with those employed in the test proper. In this way we determine not only the M.H.D. of complement, but we detect any excessive anticomplementary action of the antigen. In the test itself it is usual to employ 3 M.H.D. of complement, if this reagent is to be held constant. The use of dried complement, or of complement preserved in acid saline (Richardson 1941), adds considerably to the convenience of the test.

Precipitation Reactions, including the Kahn Test.—From our knowledge of the antigen-antibody reactions in general, we should expect that any reaction which

of the disease. It is probable that a small amount of toxin can cause extensive damage in these tissues.

Pathogenicity for Lower Animals. Diphtheria is not a natural disease of lower animals. There is a popular belief that cats may become infected and disseminate the bacilli, but this is not true. Both the local and general symptoms of human diphtheria in man can, however, be reproduced by animal inoculation. Inoculations upon the healthy mucous membrane of most adult animals lead to no changes, but if young animals be injected intratracheally, or if the mucous surface be injured before inoculation, a characteristic false membrane is produced which is histologically identical with that found in man.

The subcutaneous inoculation of a guinea pig with a sufficient amount of a young broth culture or toxic filtrate will produce death in one to four days, the time depending upon the size of the inoculum. The animal becomes obviously ill twelve to eighteen hours after inoculation, and nephritic symptoms, paralytic manifestations and other characteristics of human diphtheria are often observed. Postmortem findings include an edema and possibly necrosis at the site of inoculation, congestion of the regional lymphatics and abdominal viscera, a pleural exudate and, characteristic of diphtheritic toxemia in this animal, an enlarged and hemorrhagic condition of the adrenals. As a rule, the bacilli remain localized and are not found in large numbers in the internal organs of the infected animal. Guinea pigs that receive smaller doses and do not die by the fourth day may develop paralytic symptoms and cachexia and die later on, a condition obviously different from the acute toxemia.

Animals vary considerably in their susceptibility to infection. Rats and mice are relatively refractory, rabbits are less susceptible than guinea pigs, cats, dogs and horses. *Peromyscus* has been found to be susceptible. *Peromyscus* has been found to be susceptible to diphtheria toxin and may be used instead of guinea pigs for the virulence test.

Bacteriological Diagnosis of Diphtheria. To establish a diagnosis of infection with diphtheria bacilli, in either case or carrier, the bacillus must be isolated and its toxigenicity demonstrated. The specimen is taken on a swab, either plain or previously dipped in sterile horse serum which is coagulated on the surface by twirling in a flame. It is best to inoculate two media, Löffler's serum agar and a tellurite medium such as chocolate tellurite agar; if only a single medium can be used, tellurite is preferable. A blood agar plate should be inoculated as well, both for the isolation of diphtheria like colonies and to provide for the cultivation of hemolytic streptococci which may be present. After the plates have been inoculated a smear may be made by rolling the swab on a slide, and stained with alkaline methylene blue, it will serve to show the presence of the spirochetes and fusiform bacilli of Vincent's angina should these be present.

Diphtheria bacilli grow up in eighteen to twenty four hours' incubation. If the characteristic black or grey colonies appear on tellurite, smears may be made from such colonies and from the Löffler slant for microscopic examination, the morphology of the diphtheria bacillus is frequently not characteristic on

¹¹ Cf. Frohisher, Parsons and Tung. Amer. Jour. Hyg., 1942, 35: 351. Tung. *ibid.*, 1945, 41: 57.

replace effectively the crude tissue extract in most of the current serodiagnostic tests for syphilis (see, e.g., *Blumberg et al.* 1950, *Price and Wilkinson* 1950, 1952, *Price* 1953).

The Interpretation of the Diagnostic Serum Reactions.—This is a problem that requires the closest co-operation between the clinician and the clinical pathologist. It is considered at length in many of the reports and papers referred to above; in this book we can give only a brief discussion.

Firstly we must note the methods employed in recording and reporting results. The more common tests do not yield a numerical result, in terms of titre, dilution or ratio.

Often the range of strongly positive to doubtful and negative reactions is recorded by an equivalent range of plus signs, $++$, $+$, \pm and $-$. This notation is useful but confusing, because each serologist has his own interpretation of the different degrees in the scale. The same applies to a verbal scale, such as "strongly positive," "positive," "weakly positive," "doubtful" and "negative." Ambiguity is diminished and the clinician probably well enough served when a restricted notation, consisting solely of "positive," "doubtful" and "negative" is used (*see Cumming et al.* 1935).

The way out of the difficulty would be to specify the results obtained with each method in terms of a standard antigen, and of standard preparations of syphilitic sera giving the various degrees of positive reactions. As noted above, the first steps in this direction have already been taken on an international level.

In the absence of tests against such standards, we may get some idea of the relative sensitivity and specificity of various tests from limited comparative tests that have been made. In 1928, under the auspices of the Health Organization of the League of Nations, sera from several hundred persons clinically diagnosed as suffering from syphilis, and several hundred diagnosed as non-syphilitic, were submitted to various serological tests carried out at Copenhagen by skilled pathologists from different countries (*see Report* 1929).

The significant figures taken from this report are tabulated, in a slightly modified form, in Table 165. The various modifications of the Wassermann reaction employed, and the various workers using this test, are designated as W.R.1, W.R.2, etc. The results obtained by the Kahn test are labelled Kahn 1 and Kahn 2. Other forms of precipitation tests are labelled P.1, P.2, etc. The results are recorded as "positive," "doubtful" or "negative." It will be seen that the reports on the clinically syphilitic cases vary rather widely, and that some of the workers recorded rather a high proportion of positive or doubtful reactions among the clinically non-syphilitic cases. Taking the results at their face value, and allowing for the fact that many of the clinically syphilitic cases were clearly not in a stage in which they would give a positive serum reaction, W.R.3 with 42.6 per cent. of negatives among the clinically syphilitic, and 97.2 per cent. among the non-syphilitic has given satisfactory results. Both series of Kahn tests do better than this, and so does P.2.

The variability of the results with sera from clinically syphilitic patients, and the rather high proportion of negative results, are not surprising; because all stages and types of syphilis were included, and it is well known that the proportion of positive reactions varies widely in different stages and types of the disease. Boas, for instance (*see Reports* 1918b, 1919), records 59 per cent. of positive results in primary syphilis, 90 per cent. in secondary syphilis, 84 per cent. in tertiary syphilis, 72 per cent. in tabes, and 99.3 per cent. in general paralysis of the insane. It is in the type of case that shows a relatively low proportion of positive reactions,

tellurite as indicated above. It is often inferred that only diphtheria bacilli grow as black colonies on tellurite medium. This is not true, for any bacterium that reduces tellurite will produce similar colonies; tellurite-reducing bacteria, other than diphtheria bacilli, from the nose and throat are usually staphylococci or micrococci and as a rule their colonies resemble those of the *mitis* variety of diphtheria bacillus but are blacker.

If morphologically typical bacilli are found toxigenicity must be tested by animal inoculation. This is ordinarily carried out in the guinea pig by subcutaneous or intracutaneous inoculation. In the first instance the growth from a Löffler slant is suspended in 10 ml. saline and 4 ml. injected subcutaneously into each of two guinea pigs, one of which has received 250 units of diphtheria antitoxin twenty-four hours previously. The diphtheria bacillus will kill the unprotected pig in three to five days and autopsy will show local edema and the characteristic hemorrhagic enlarged adrenals, while the protected animal will survive. For the intracutaneous test the growth from a Löffler slant is suspended in 20 ml. saline and 0.15 ml. injected into the shaven abdominal skin of each of two pigs as above. Toxigenicity is indicated by the development of a local infiltrated lesion which shows superficial necrosis in two or three days in the unprotected pig. By the latter technique a number of tests may be carried out in the same pair of animals.

The virulence test may also be carried out in the rabbit. The growth from a Löffler slant culture is suspended in 2 to 3 ml. of sterile infusion broth, and 0.1 ml. injected intradermally. Four hours later the animal is given 1000 units of antitoxin intravenously, and immediately a second intradermal inoculation of 0.1 ml. of the bacterial suspension.

Reactions should be read at 72 hours. If the bacteria is toxigenic, the site of the first inoculation will be a central necrotic area, usually hemorrhagic, surrounded by a zone of erythema. The inoculation of antitoxin does not affect a reaction to the first inoculation, but does specifically inhibit a reaction to the second inoculation, and the site of the latter appears as a small, pinkish papule. Eight to ten such virulence tests may be carried out simultaneously in the same animal.

Immunity. Immunity to diphtheria, arising as a consequence either of recovery from a frank attack of the disease or of inapparent infection, is essentially an antitoxic immunity. Antibacterial substances appear to be of little significance and the refractory state is associated with the presence of antitoxin in the blood serum and body fluids.

The Schick Test. Immunity to diphtheria, then, may be measured by the amount of circulating antitoxin present in a given individual. A skin test has been devised by Schick, and is known as the Schick test, in which a minute amount of diphtheria toxin is injected intradermally. In the non-immune the irritant action of the toxin gives rise to a local erythema followed by necrosis and desquamation, and the reaction is said to be positive. In the immune, however, the toxin is neutralized by the antitoxin that is present, the characteristic reaction does not develop, and the reaction is negative. The amount of toxin injected is usually 1/50 of a guinea-pig MLD in a volume of 0.1 or 0.2 ml.; the Permanent Standards Committee of the League of Nations specifies 1/40

Reactions in both yaws and pinta are generally regarded as true positive reactions, indicating treponemal infection.

False Positive Reactions.—There is another class, often described as "biological false positive" reactions, to distinguish them from false positives due to faults in the serological technique. For example, patients with leprosy, malaria, sleeping sickness, tuberculosis and other febrile diseases, and pregnant women, may be positive. Table 167 displays some of the results from a trial made by the Public Health Service of the United States; it includes the syphilitic and the normal subjects from Table 166. It will be noted that the percentage of positives is high in leprosy, lower in malaria (see also Cox and Durant 1945), and very small in febrile conditions and pregnancy. Another point that emerges from the figures, typical of many serological tests for syphilis, is that the high percentages of true and "false" positives are associated (e.g., W.R. 3, Kahn 2, P 2 and P 5); i.e., the more sensitive the test, the less specific it is.

TABLE 166
RESULTS OF COMPARATIVE TRIALS OF DIFFERENT COMPLEMENT-FIXATION AND PRECIPITATION TESTS

	Percentage of Sera reacting positively.			
	Untreated Primary Syphilis.	Untreated Secondary Syphilis.	Late Syphilis Varying Treatment.	Normal. Non-syphilitic.
W.R. 1	53.7	100	66.6	0.0
" 2	65.9	100	72.1	0.0
" 3	82.5	100	86.4	0.7
" 4	69.8	100	58.0	0.0
Kahn 1	76.7	100	76.9	0.0
" 2*	82.9	100	84.3	3.3
" 3*	80.5	100	83.0	0.7
P. 1	72.1	100	82.4	2.0
" 2	81.0	100	84.5	0.7
" 3	58.1	98.4	64.5	3.3
" 4	74.4	100	71.5	0.0
" 5	72.1	98.5	83.6	1.3
" 6	70.7	98.4	63.1	0.7

* See text.

False positives are also reported in respiratory infections, infectious mononucleosis, virus diseases such as measles and vaccinia (Rein and Elsberg 1945), and more rarely in a number of other diseases (see Stokes and James 1949). The globulin responsible for the false reaction differs in some respects from the syphilitic antibody, particularly in being inhibited by a lecithin associated with the globulin fraction of human serum (see Volkin *et al.* 1947, Volkin 1949). The origin of these antibodies is obscure. Kahn (1949) describes a "universal" flocculation reaction with his standard antigen when it is used at another salt concentration—a reaction which is positive in the sera of normal men and animals. He regards the antibody as the result of auto-immunization by lipids released during ordinary tissue breakdown, when the breakdown is intensified in diseases like leprosy or malaria, the "universal" antibody is abundant enough to react in the conditions of the usual serological tests for syphilis.

Treponema Immobilization (T.P.I.) Test.—Nelson and Mayer (1949) and Nelson and Diesendruck (1951) described the conditions for detecting an antibody acting directly on *Trep. pallidum*. Spirochaetes are harvested by mincing heavily infected rabbit testicle, and the resulting suspension is preserved in a specially devised medium. They are incubated at 35° C. with guinea-pig complement and the serum under test, in an atmosphere

MLD in 0.2 ml. and 1/50 MLD in 0.1 ml.²³ According to Moloney and Taylor,²⁴ however, a toxin of three times this strength gives more sharply defined reactions. Considerable interest has attached to diluents for the Schick toxin since it is not stable in phenol-saline solutions. The dilute toxin is, however, stable in 2 per cent peptone solution, a borate buffer-gelatin solution, and a glycerol-gelatin solution which has been proposed recently. The advantages of a ready-diluted Schick toxin are obvious, and toxins diluted ready for use are now generally available.

For many years a negative Schick test has been regarded as indicating the presence of 1/20 unit or more of antitoxin per milliliter in the blood serum and a positive test less than 1/40 unit. More recent experiments, however, have indicated that the so-called "Schick level" of immunity is much lower than this and in the neighborhood of 1/250 to 1/500 unit of antitoxin; negative reactions have been obtained in persons with as little as 0.0005 unit. Phair²⁵ has expressed the opinion held by a number of workers that a negative Schick test is indicative not only of antitoxin content of the blood but also involves a defense mechanism other than that of antitoxin production.

A scarification test in which diphtheria toxin is introduced by punctate scarification rather than intradermal injection has been introduced by Reh and is called *Reh's test*. It is said to be somewhat simpler to perform than the Schick test and, when carried out with a potent toxin (with a guinea-pig MLD of 2000 per ml.), to give parallel results with the Schick test.

The question of whether the Schick test is indicative of a degree of immunity such that subsequent infection is highly improbable is one that cannot be answered *a priori*. Experience has shown, however, that the assumption that a Schick-negative person is, for all practical purposes, immune, is pragmatically sound.

Prophylactic Immunization. It was early observed that experimental animals can be immunized to diphtheria by the injection of living cultures of the bacilli after a protective dose of antitoxic serum or by the inoculation of toxin neutralized with antitoxin. Theobald Smith suggested the use of toxin-antitoxin mixtures in the immunization of horses in 1907, and the same method was used by von Behring in 1913 to immunize children. The use of toxin-antitoxin for the immunization of man, however, was developed largely through the efforts of Park in New York City from 1913 onwards.

Toxin-Antitoxin. The mixture usually used contains 0.1 L₁ dose of toxin per milliliter. The toxin is slightly underneutralized (5 ml. of the mixture should produce diphtheritic paralysis in 300 gm. guinea pigs) but depends for its immunizing efficiency not on the slight excess of toxin but on a slow dissociation of the toxin-antitoxin complex to liberate free toxin. Administered in 3 doses of 1 ml. each at intervals of one to two weeks, toxin-antitoxin produces an immunity in 85 per cent of individuals inoculated. The immunity

²³ Report of the Permanent Commission on Biological Standardization, League of Nations Health Organization, London, 1931.

²⁴ Moloney and Taylor, *Jour. Immunol.*, 1937, 33, 191. See also Cameron and Giffard, *Canadian Jour. Pub. Health*, 1941, 32, 83.

²⁵ Phair, *Amer. Jour. Hyg.*, 1942, 36, 283.

The use of the Wassermann reaction in the control of treatment is too complex to be considered here. Broadly, it may be stated that, in a case which comes under treatment early, and which is efficiently treated, the Wassermann reaction becomes negative and remains so; whereas in a case which escapes treatment during the early stages, or which is inefficiently treated during this period, it is extremely difficult, if not impossible, to produce a permanently negative Wassermann reaction by treatment administered several years after infection has been contracted (Browning and Mackenzie 1924, Report 1926).

It may be added that the tests used in the examination of the blood serum may also be applied to the cerebrospinal fluid in suspected cases of neurosyphilis; though certain of the precipitin tests give rather poor results when used for this purpose (see Cumming *et al.* 1935). Such examinations should, of course, be controlled by examination of the fluid for cells, proteins, type of colloidal-gold curve and so on.

Diagnostic agglutination tests with suspensions of spirochaetes have been described (Tani 1940, Cain 1953), but their value in practice has still to be established.

Prophylaxis and Treatment.

We do not propose to discuss the various hygienic methods that have been advocated for the prevention and control of syphilis.

Bacteriological methods of prophylaxis do not exist. Animal experiments indicate that it is difficult or impossible to protect animals by vaccination with dead spirochaetes against subsequent infection with living organisms (Uhlenhuth and Mulzer 1913, Grossmann 1929, Eagle and Fleischman 1948, Magnuson *et al.* 1947), though McLeod and Magnuson (1953) record the formation of immobilizing antibodies in these circumstances. Antibodies have been demonstrated in the blood of infected animals, but protective antibodies, when demonstrable, are feeble (Turner *et al.* 1948). Metchnikoff and Roux (1901a) obtained some evidence that apes inoculated with a weakly virulent strain of syphilis were protected against subsequent inoculation with a fully virulent strain, but the results were not conclusive. Treatment with organic arsenicals, either arsphenamines like salvarsan, or oxophenarsine (mepharsen), is successful in curing the disease in the early stages, but once the secondary stage has passed, definitive cure appears to be impossible.

In communities where it is available, penicillin has largely replaced the arsenicals. Its effect in man was first demonstrated by Mahoney, Arnold and Harris (1943). Benzylpenicillin is the most effective (Eagle 1946). In man it is estimated to be 2-4 times as effective as oxophenarsine (Eagle *et al.* 1946).

Prophylaxis in the rabbit, in which it is estimated that the spirochete divides about once every thirty hours (Magnuson *et al.* 1948), is possible with concentrations of the drug in the serum of the same order as those that are treponemocidal *in vitro* (Eagle *et al.* 1950), though more is required when large inocula are used. For cure of the rabbit, the dose increases with the size of the inoculum, and with the time between infection and administration. It is constant for 4 days, and rises to 30 times this amount after 6 weeks (Eagle *et al.* 1947, Eagle 1949). There is little doubt that penicillin acts synergically with arsenic or bismuth preparations in the experimental animal (Magnuson and Rosenau 1948, Kolmer 1947, 1948, 1951), and combined treatment has been practised in man, especially in relapse cases (Harrison 1945, Report 1946). Large doses totalling 3-6 hundred thousand units of "depôt" preparations of penicillin alone, which ensure effective concentrations of the drug in the blood for long periods, are curative in about 90 per cent. of human cases of early syphilis within a few weeks (see Thomas 1948, Arnold *et al.* 1952). As with bismuth and the arsenicals, late syphilis is more resistant to cure. There is little doubt that true relapses occur, usually within six months of stopping treatment. But the short period of treatment, with the rapid cure of local lesions, and the reputation of penicillin as a cure,

develops slowly, and one to six months may be required for the Schick reaction to become negative. Accidents may occur as a consequence of dissociation of the toxin-antitoxin mixture—freezing in one instance produced such dissociation—but these are rare, particularly with the 0.1 L₊ dose mixture. There is, of course, the possibility of sensitization of the inoculated individual to horse serum.

Toxoid. The use of formol toxoid or anatoxin as an immunizing agent was introduced by Ramon in 1923 and has been widely adopted. As pointed out elsewhere (p. 204), toxin treated with formaldehyde (in this case a potent toxin of more than 15 Lf doses per milliliter is incubated with 0.3 to 0.4 per cent formalin at 37° C. for one month) loses its toxicity but retains its antigenicity and is a highly efficient immunizing agent. The administration of this material in three doses of 0.5, 1.0 and 1.0 ml. at intervals of two to three weeks renders 95 per cent of persons Schick-negative. It was at first thought that toxoid might entirely replace toxin-antitoxin as an immunizing agent, but this has not proved to be the case. Reactions to the bacillary protein, while not of great importance as a rule in young children, may be relatively severe in older persons, and its use is best restricted to children under twelve years of age. Reactivity may be tested for by the intradermal injection of toxoid—the *Moloney test*.

Toxoid-antitoxin floccules (the precipitate coming down at the optimal antigen-antibody ratio) have been used in England to a considerable extent. There is, presumably, a partial purification of the toxoid by precipitation with antibody.²⁶ This material and toxin-antitoxin floccules have not been widely used in the United States. Toxoid precipitated with protamine appears to be an effective immunizing agent without giving the untoward reactions sometimes observed with alum-precipitated toxoid.²⁷ Pillemer and Toll²⁸ have produced highly purified toxoid by methanol precipitation in the cold which gave 2000 or more Lf per mg. nitrogen, but this material has not as yet been adequately tested as an immunizing agent.

Alum-Precipitated Toxoid. It has been found that toxoid precipitated with potassium alum (small amounts, 1 to 2 per cent, are required) is superior as an immunizing agent to ordinary formol toxoid. Present preparations are treated with charcoal prior to alum precipitation to remove color and extraneous nitrogenous material.²⁹ The precipitate is insoluble (it may be redissolved in sodium citrate or sodium tartrate) and remains in the subcutaneous tissue for a considerable period of time, thus providing a prolonged antigenic stimulus. It was first thought that a single injection of this material was sufficient to provide a solid immunity; some of the early reports indicated that 90 to 95 per cent of Schick-positives became Schick-negative as a consequence of a single injection. The administrative advantages of a single injection are, of course, obvious. It has become increasingly clear in recent years, however, that a single injection is not sufficient; as little as 11 per cent conversion has been

²⁶ For a discussion of this material see Watson, Taggart and Shaw: *Jour. Path. Bact.*, 1941, 53:63.

²⁷ Ross: *Amer. Jour. Dis. Children*, 1944, 68:172.

²⁸ Pillemer and Toll: *Science*, 1947, 105:102.

²⁹ For details of preparation see Barr, Pope, Glennly and Linggood: *Lancet*, 1941, ii:301.

in its typical form only amongst the dark-skinned races. The causative organism, *Treponema pertenue*, was discovered by Castellani in 1905. It is a spirochete morphologically indistinguishable from *Trep. pallidum*. Yaws occurs particularly in childhood. The commonest site for the primary lesion is on the lower extremities. The secondary eruption usually appears 2-4 weeks later. In the late stages of yaws, bone lesions and ulcerative skin lesions develop. Yaws does not appear to be acquired congenitally. It is probably spread by contact and may be transmitted by insects. The disease is not as a rule of venereal origin. It is particularly prevalent in environments that have a high humidity.

Wassermann reactivity follows the same course as in syphilis. Yaws is curable by the arsenicals, and readily so by penicillin. (For information about the epidemiology, treatment and control of yaws, see Symposium 1953.)

Pinta, like yaws, is a contagious, inoculable disease, and is notably prevalent among coloured people in the American tropics. The characteristic lesions are papules that coalesce to form mottled, depigmented lesions on the skin, mainly on the extremities. The disease occurs at any age; it is non-venereal, and spreads by contact, and possibly by insects. The causative organism, *Trep. carateum*, was first demonstrated by Saenz and his colleagues (1938). It is morphologically indistinguishable from *Trep. pallidum*. Wassermann and flocculating antibodies appear in a low proportion of patients in the primary stage, in about 60 per cent. of those in the secondary stage, and in most of those in the late stages. Arsenical drugs, and (see Rein *et al.* 1952) depôt penicillin are curative.

Much remains to be done to establish the exact relationship, bacteriological and immunological, of *Trep. pertenue* and *Trep. carateum* to *Trep. pallidum*. *Trep. pallidum* and *Trep. pertenue* cross-react with the corresponding immobilizing antibodies in rabbits (Khan *et al.* 1951; see also Varela and Palencia 1954). There are suggestions in man of a cross-immunity. Thus, Parham (1922) in Samoa, and Wilson and Mathis (1930) in Haiti, concluded on epidemiological grounds that yaws in childhood protected against syphilis in adult life. Cross-immunity between syphilis and pinta is less good.

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reported. There is also a tendency for such Schick-negatives to revert to Schick-positives within a year or two, though why this should be the case is not at all clear. It seems established that a single injection is not sufficient but two injections provide a solid immunity. The primary dose should be not less than half the total toxoid given and may be as great as two-thirds of it.³⁰ Alum toxoid has the same tendencies to produce untoward reactions in older persons that are observed with formol toxoid.

Passive Immunity. Susceptible, *i.e.*, Schick-positive, individuals may be passively immunized to diphtheria by the injection of antitoxic horse serum or purified preparations of antitoxin. Such immunity is of relatively short duration and is not effective for longer than two or three weeks at the most. Passive immunization is not so extensively practiced now as it formerly was but *is, of course, indicated in the case of susceptible individuals who are directly exposed to the disease.* Except on a small scale, as in a hospital ward, it is not practical to control epidemic diphtheria through passive immunization.

SUSCEPTIBILITY OF VARIOUS AGES TO DIPHTHERIA

(As Indicated by the Schick Test)

Age	Susceptible, Per Cent
Under three months	15
Three to six months	30
Six months to one year	60
One to two years	70
Two to three years	60
Three to five years	40
Five to ten years	30
Ten to twenty years	20
Over twenty years	12

The use of combined active-passive immunization in which both toxoid and antitoxin are given simultaneously, the former in protective amounts, has been of some interest. More recent work indicates that the passive protection conferred by antitoxin does not interfere seriously with the immune response though there is a period of low immunity, after the second or third week, after the passive protection has been exhausted. A second inoculation of toxoid is, of course, highly desirable.³¹

The Therapeutic Use of Antitoxin. Serum therapy in diphtheria is more successful than in any other disease, and there is no question of its efficacy in reducing the case fatality rates. As in the case of tetanus and botulism, the therapeutic administration of antitoxin cannot bring about repair of tissues already damaged by toxin. Early administration is, therefore, essential, and there is progressive increase in the case fatality rate with each day's delay. Park advises in mild cases 3000 to 5000 units, in moderately severe cases 10,000 units, and in severe toxic cases 20,000 units or more in adults and 10,000 to 20,000 in children. There is no limit, beyond the volume, to the number of units that may be safely injected. Antitoxin is generally administered intra

* Cf. Bousfield Brit. Med. Jour., 1913, p. 706.

³¹ See Downie, Glenn, Parish, Smith and Wilson Brit. Med. Jour., 1941, p. 717, Phair and Ross Amer. Jour. Hyg., 1942, 35:377.

muscularly but in severe cases may be given intravenously. It is completely ineffective when given by mouth.

Of considerable practical significance is the concentration of antitoxin, since the volume injected is a limiting factor. Usually horse serum contains 500 to 700 units per ml. and exceptionally 1000 to 1500. Concentration of the antitoxin by salting out and other procedures is generally practiced, for, although some antitoxin is lost in the process, the concentration is increased with a corresponding reduction in the volume to be injected.

Epidemiology.³² The epidemiology of diphtheria is considerably better understood than that of any other disease, in part because the causative agent can be isolated with relative facility from infected individuals, and in part

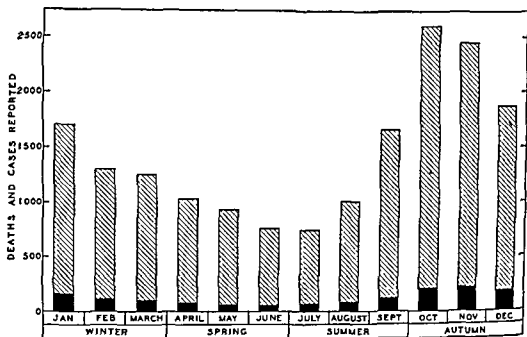


Fig. 142. The seasonal incidence of diphtheria. Averages of reported cases by months for the years 1939 to 1945 inclusive. Data from Supplements to Public Health Reports.

because the Schick test allows the differentiation of the immunes and the non-immunes. As in the case of other respiratory diseases, infectious material leaves the body in the secretions of the nose and throat, is transmitted from man to man by contact or infective droplets, and enters the body via the mouth and nose. Furthermore, the diphtheria bacillus is disseminated not only by persons with the disease but also through the agency of healthy carriers in whom there is no clinical evidence of infection. Unlike many of the diseases of the respiratory tract, however, diphtheria is an immunizing disease and prolonged or repeated contact with the bacillus frequently results in the development of a solid immunity to the disease in its clinical manifestations.

Immunity and Susceptibility. Schick testing indicates that while susceptibility is low in the first six months of life, the proportion of Schick-positives increases rapidly and is at a maximum in children under four or five years of age, then gradually declines until 80 per cent or thereabouts of adults are

³² For a critical discussion of the epidemiology of diphtheria during the past forty years see Russell. Med. Res. Council (Great Britain), Spec. Rept. Ser. No. 247, 1943.

Japan, where in 1933 there were as many as 1,636 cases. The United States seems to suffer but little. In Europe, Holland and France appear to have the highest incidence rate, but statistics of the real prevalence of the disease are unobtainable (see Report 1934). In Great Britain it is endemic in sewer workers (Fairley 1934, Alston and Brown 1935, Stuart 1939) and in those engaged in washing fish (Davidson *et al.* 1934, Davidson and Smith 1936, Smith and Davidson 1936, Smith 1949).

The incubation period of the disease is 5 to 7 days as a rule, rarely as long as 13 days (Inada 1917). In the first or febrile stage, which lasts for 6 or 7 days, there is high fever, conjunctival congestion, muscular pains, and albuminuria. In the second or icteric stage, which lasts from the 7th to the 13th day, jaundice appears, there is a tendency to hæmorrhage, and death may occur. The third stage, or stage of convalescence, is marked by the gradual subsidence of the jaundice and other symptoms; it may be interrupted, however, by a secondary fever (Inada 1917).

The disease varies considerably in its symptomatology, depending to some extent on the nature of the causative organism. In Great Britain most cases are caused by *Lepto. icterohæmorrhagiæ*, but 10–15 per cent. or so follow infection with *Lepto. canicola* (see Chapter 38 and Broom 1951b). The disease caused by *Lepto. icterohæmorrhagiæ* tends to be more severe, jaundice occurring in about 70 per cent. of recognized cases; nephritis is sometimes the dominant symptom. In the form caused by *Lepto. canicola* jaundice is present in only about 18 per cent. of cases. Serous meningitis may occur in either form, but is much commoner in that due to *Lepto. canicola*. The case-fatality rate varies in different outbreaks. Cases without jaundice are practically never fatal, and in the canicola form of the disease even cases showing jaundice almost invariably recover. In outbreaks and sporadic cases caused by *Lepto. icterohæmorrhagiæ* the fatality rate in Japan varies from 4·6 to 32 per cent. (Inada *et al.* 1916, Report 1934); on the western front during the 1914–18 war it was under 6 per cent. among the British and 13 per cent. among the German troops (Stokes *et al.* 1917, Uhlenhuth and Fromme 1918); in the Scottish outbreak it was about 25 per cent. (Buchanan 1927); and in the civilian population of England and Wales it is about 15 per cent. (Broom 1951a).

In man the spirochætes are widely distributed in the body during the 1st week of the disease, and can generally be demonstrated in the blood by guinea-pig injection, and occasionally by microscopical examination. After the 7th to 9th day they leave the blood and appear in the urine, at first in very small numbers, but gradually increasing till during the 3rd and 4th weeks they can be found microscopically by dark-ground illumination in the majority of cases. They persist in the urine for a variable time, but can rarely be found after the end of the 5th week. In patients dying during the febrile stage of the disease spirochætes can be demonstrated microscopically in the kidney, liver, adrenals, spleen, testicle, lymphatic glands, voluntary and cardiac muscle, and arterial walls; less frequently in the lung, pancreas, intestine, nervous system, and skin (Inada *et al.* 1916). If death occurs during the 2nd week or later, the chief organ in which spirochætes are found is the kidney; in the other organs they are less numerous or absent.

Occupational Incidence.—Weil's disease occurs chiefly in damp, badly drained, rat-infested situations. Thus in Japan it was noticed that the disease occurred in coal mines; that only the miners who worked in a particular section of the mines were affected; that this section was under water; and that when the water

Schick-negative. (See the accompanying table.) The initial immunity of the very young is passively transferred from the mother and is not of long duration. The increase in the proportion of immunes, however, is by no means entirely a result of recovery from clinical diphtheria, and the question arises as to how these individuals acquire an active immunity.

Carriers. As indicated above, healthy individuals may harbor virulent diphtheria bacilli in their throats. These carriers need be neither immunes nor convalescents and are, for the most part, casual carriers. There is no precise information concerning the duration of this transient carrier state; it may possibly be about two weeks. The proportion of carriers has been investigated by a number of workers. In a study of Baltimore school children, Doull and Fales³³ found an average carrier rate of 2.32 per cent from November to May. On the basis of this Frost³⁴ has estimated the carrier incidence in the five to

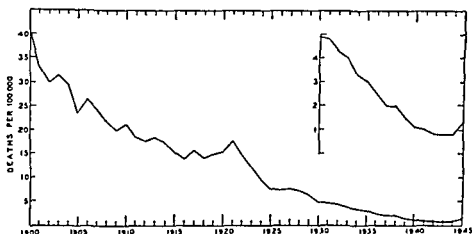


Fig. 143. The prevalence of diphtheria in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census

fourteen age group in that city to be 2538 per 10,000. At this rate 75 per cent of the population becomes infected at least once in five years, 95 per cent in ten years, and over 99 per cent in fifteen years, while very considerable proportions would suffer repeated infections, the average being 2.5 infections per person in ten years. Others have recorded considerably higher carrier rates. Dudley³⁵ has reported 6.6 per cent in a boys' school, and repeated swabbings showed that at least 40 per cent carried the diphtheria bacillus at one time or another during the yearly period.

There is, it appears, ample opportunity for contact with virulent diphtheria bacilli, and there is every reason to suppose that the increasing proportion of Schick negatives in the progressively higher age groups is a consequence of an active immune response to the presence of these microorganisms in the nose and throat. It may be noted parenthetically that a similar situation may very likely prevail in certain other diseases in which technical difficulties have prevented its demonstration.

³³ Doull and Fales, *Amer. Jour. Hyg.*, 1923, 3:604.

³⁴ Frost, *Jour. Pres. Med.*, 1928, 2:325.

³⁵ Dudley, *Jour. Hyg.*, 1932, 32:193.

common, from 7 to 40 per cent. of all rats examined proved to be infected (Schuffner 1934). The frequency of infection depends largely on the age of the rat. Young rats are seldom infected, but over 50 per cent. of adult rats may harbour the spirochaetes. The black rat rarely act as a carrier.

The spirochaetes are found in the kidney and the urine, not in the blood or liver. Intraperitoneal injection of rat's urine, even in small amounts, 0.1-0.2 ml., into guinea-pigs may give rise to fatal hæmorrhagic jaundice. Not all specimens of rats' urine in which leptospiræ are visible microscopically prove infective; this is probably dependent on the acidity of the rat's urine, which rapidly proves fatal to the organisms. The same applies to the effect of human urine; only in one-third of cases of Weil's disease does the urine prove to be infective. Uhlenhuth and Zuelzer (1921) found that fresh human or rats' urine rapidly killed the spirochaetes; but if the urine was neutralized, the organisms remained virulent for 2 days.

Leptospiræ in Water.—In view of the frequency of infection of rats' urine, it should be possible to demonstrate the presence of *Lepto. icterohæmorrhagiæ* in contaminated water.

Spirochaetes morphologically identical with *Leptospira icterohæmorrhagiæ* are widespread in water. They were first observed by Wolbach and Binger in 1914, and have since been found by numerous workers in different parts of the world. In this country and in America they are known as *Leptospira biflexa* and in Germany as *Spirochæta pseudocitrogenes* (see Chapter 38). As pointed out by Zuelzer (1928) they are generally found attached to other spirochaetes and protozoa. They are especially prevalent in the slime of ponds, lakes, and rivers, in the slime that collects on the ends of water taps and pipes, and in the roof slime of mines. They are very susceptible to acid and are therefore confined to waters with a pH of over 6.8. Thus they are abundant along the east coast of Sumatra where the water is alkaline and practically absent from Java where the water is acid (Sardjito and Zuelzer 1929). They are also susceptible to salt, and are said to perish in 3 days in alkaline water containing 0.17 per cent., and in a few hours in water containing 1.7 per cent., of chlorine as chloride (Schuffner 1934).

These organisms have occasioned much discussion. For some time opinion was influenced by the experiments of Baermann and Zuelzer (1927, 1928). These workers brought evidence to suggest that, though on first isolation water leptospiræ were avirulent, by passage through guinea-pigs, or occasionally through human beings, they could be so raised in virulence as to give rise in the guinea-pig to typical hæmorrhagic jaundice. In fact Baermann and Zuelzer regarded *Lepto. biflexa* merely as an avirulent form of *Lepto. icterohæmorrhagiæ*. Extensive observations by various workers have failed to confirm these findings. Possibly Baermann and Zuelzer were working with a strain of *Lepto. icterohæmorrhagiæ* that had become avirulent by residence under saprophytic conditions, or they may have isolated from one of their guinea-pigs a naturally infective strain entirely different from their passage strain. Except in special conditions, where the locality is heavily rat-infested, it is uncommon to isolate a pathogenic leptospiral strain from water. Since *Lepto. biflexa* is widely distributed over the globe, while cases of Weil's disease are topographically and occupationally circumscribed, it is difficult to avoid the conclusion that *Lepto. biflexa* has no ætiological relationship to the disease. Most of the evidence suggests that water becomes contaminated with *Lepto. icterohæmorrhagiæ* from rats' urine, and that even in favourable circumstances the organisms die out fairly rapidly. For this reason infection of human beings is unlikely except from water exposed to frequent and heavy contamination.

Summarizing, it may be said that the evidence points strongly in favour of infection occurring through contact, often close and prolonged, with water or

It may be asked why contact with virulent diphtheria bacilli does not result in clinically apparent infection more often. The production of disease is, of course, dependent upon the balance between virulence and resistance as pointed out in an earlier chapter (Chap. 8). Climate appears to be of some importance, for the proportion of Schick-negatives in tropical countries is quite as high as in the temperature zones yet clinical diphtheria is much less common. Racial factors may be involved also, for in the Baltimore studies the carrier rate in Negroes was not significantly different from that in whites, yet the morbidity rate for the former was much lower.

The opportunities for the transmission of infection from person to person are reflected in both the morbidity and the mortality rates. There appears, for example, to be a direct relation between school attendance and the incidence of diphtheria, and the deaths from this disease tend to be concentrated in the preschool age group with increasing urbanization.

The Control of Diphtheria. It will be obvious from the above considerations that diphtheria is widely disseminated in the human population and cannot be controlled by the isolation of carriers or, except in a strictly limited sense, by quarantine of cases. The control of diphtheria is entirely a matter of immunization and, if a sufficiently large proportion of the susceptible population is rendered immune, the prevalence of clinical diphtheria should decrease. Godfrey³⁶ has found that the immunization of 50 per cent or more of the children of school age, five to fourteen years of age, did not produce a fall in the incidence of diphtheria in a number of large American cities, but that when 30 per cent or over of the preschool children were immunized there was a definite reduction in the incidence of diphtheria not only among these children but in the community as a whole. Immunization confers a marked, though not absolute, protection in the individual case, of course, and it would appear best from both the individual and group points of view that immunization be effected early.

To what extent prophylactic inoculation and the therapeutic use of antitoxin have influenced the decline in diphtheria shown in Fig. 143 is problematical. The disease was endemic during the first half of the nineteenth century though showing increasing epidemic tendencies. Between 1850 and 1860 a great pandemic developed, apparently from a focus in France, which swept over the world. A high mortality was maintained for twenty-five or thirty years, then around 1885 a decline set in which continued to about 1941. Immunization was not generally practiced until about 1920, though antitoxin therapy began somewhat earlier. Russell³² is of the opinion that the decline in certain areas, such as New York, in which active immunization has been extensively applied is more rapid than could be expected from the trend of pre-immunization years, and attributes this increase to prophylactic inoculation.

Since 1941 there has been a general increase all over the world in the prevalence of diphtheria. It apparently began in Germany in 1939, possibly due in part to mass movement of children into camps without adequate immunization, with a doubling of the already high (285 and 207 in Austria and Germany) morbidity rate, and an increase in severity as indicated by increase in case fatality rates of 3.8 per cent in 1937-38, to 4.4 per cent in 1939, and 5.0 per cent in 1940. The disease spread into neighboring countries in northwest

³⁶ Godfrey: Amer. Jour. Pub. Health, 1932, 22:237.

SUMMARY OF THE COMMONER FORMS OF HUMAN LEPTOSPIRAL INFECTIONS (Modified considerably from Walch-Sorgdrager 1939).

TABLE 168

Disease	Clinical Symptoms	Epidemiological Features	Causative Organism	Animal Inoculation	Carrier
Well's	Jaundice frequent Fatality variable, but often high	Affects persons brought into contact with water contaminated by rats' urine. Epidemic in sewer and mine workers. Males more often attacked than females.	<i>L. icterohaemorrhagiae</i>	Virulent for guinea-pigs producing fatal jaundice.	Rats, <i>R. norvegicus</i> and <i>R. alexandrinus</i>
Well's	Jaundice much less frequent and usually slight. Serious meningitis common. Practically never fatal.	Attacks persons brought into close contact with sick dogs.	<i>L. canicola</i>	Virulent for young golden guinea-pigs.	Dogs
Indonesian Weil's disease	Variable severity, but usually mild with infrequent jaundice.	Uncertain, but infection may be derived in Indonesia from dogs and cats as well as rats. In Italy the rice-field workers are affected in the summer.	<i>L. bataviae</i>	Moderately virulent for guinea-pigs.	Rats, <i>R. norvegicus</i> , and in Italy the field mouse, <i>Microtus minutus sordidus</i>
Eastern Weil's disease (<i>S. pyrochlorosis febrilis</i>)	Usually a short mild febrile disease with infrequent jaundice.	Mainly affects labourers on plantations. Occurs in Sumatra, Malaya, and Australia.	<i>L. pyrogenae</i>	Moderately virulent for guinea-pigs.	Field rats, <i>R. brevicaudatus</i>
Weil's disease of Andaman	Jaundice fairly frequent. Fatality low to moderate.	Affects mainly men working in rice fields and coco-nut plantations. Chiefly in autumn.	<i>L. andaman</i>	Moderately virulent for guinea-pigs.	?
Hassani and (Alayami A)	Jaundice fairly frequent. Severe fatal. Clouding of vitreous humour frequent.	Occurs in Japan, Malaya, and Indonesia. Affects workers in moist lowlands.	<i>L. autumnalis</i>	Moderately virulent for guinea-pigs, often producing jaundice.	Field mice, <i>Apodemus sordidus</i>
Nankayami or 7-day fever of Japan (Alayami B)	Jaundice rare. Disease mild. Lymph glands may swell.	Occurs in Japan and Formosa. Affects mainly labourers in fields and woods.	<i>L. hebdomadis</i>	Very low virulence for guinea-pigs.	Field mice, <i>Microtus montebellii</i>
Weil-like disease	Generally a mild fever without jaundice.	Mainly in Denmark. Infection occurs chiefly on farms in the late autumn.	<i>L. sejtroc.</i>	Virulence for guinea-pigs low.	Field mice, <i>Mus sylvaticus</i>
Cane or Coastal fever of Queensland	Jaundice rare. Glands often enlarged. Severe and sometimes fatal.	Cane-field workers in Queensland. Mainly men.	<i>L. australis</i>	Moderately virulent for guinea-pigs.	Field rats, <i>R. conatus (culmorum)</i>
Swamp fever Mud fever	Jaundice rare. Not fatal.	Endemic in Germany, Italy, Russia and the Andaman Islands. Affects mainly harvesters on swampy ground.	<i>L. grippotyphosa</i>	Virulence for guinea-pigs very low.	Field mice, <i>Eutamias glareolus</i> , <i>Microtus agrestis</i> , <i>Microtus arvalis</i>
Swamp fever disease (7-day fever of Queensland)	Jaundice rare. Not fatal.	Occurs in Australia, Indonesia, Switzerland and Italy. Affects farm workers, especially those in contact with pigs.	<i>L. pomona</i>	Virulence for guinea-pigs very low.	Endemic infection of pigs and cattle
7-day fever of Queensland	Jaundice rare. Not fatal.	Among workers with farm animals.	<i>L. muis</i>	Virulence for guinea-pigs moderate.	Endemic infection of pigs and cattle.

Europe. In Belgium the number of cases rose from 2419 in 1939 to 16,072 in 1943, in Holland there were 1273 cases in 1939, 5501 in 1941, 19,527 in 1942 and 56,603 in 1943, in Norway there were 54 cases in 1939 and 22,787 in 1943. Diphtheria was the leading epidemic disease of the war years in Europe and an important cause of death in the German army. By 1945 it had declined somewhat, but the rate in the American and British occupation zones in Germany was still 430 to 560 with a case fatality rate of 5.7 per cent, and in France and Belgium the morbidity rates were still about three times the prewar rate. Some rise was also experienced in the United States, from 13,744 cases reported in 1943 to 17,612 in 1945, and in the large cities the mortality rate rose from 0.56 in 1941 to 0.88 in 1946.³⁷ The sharply increased prevalence of a more severe diphtheria has led to some speculation as to the possible appearance of unusually virulent strains but there has been no bacteriological evidence in support of the suggestion. It seems more likely that the increased prevalence is attributable to some degree of breakdown in the application of artificial immunization, due in part to apathy and in part to population displacements coincident with war, but in general it has not been adequately explained. Such a phenomenon serves as a timely reminder that, though many of the infectious diseases have been brought under some degree of control, that control must continue to be exerted.

THE DIPHThEROID BACILLI

form occurring in the human throat and readily confused with the diphtheria bacillus on microscopic examination was first observed by Löffler and by von Hofmann Wellenhof. It is known as *Hofmann's bacillus*, *Corynebacterium hofmanni*, or *Corynebacterium pseudodiphtheriticum*. It differs slightly from the diphtheria bacillus in that it is somewhat shorter and plumper and does not ferment dextrose. Most important, it does not form a soluble toxin and is readily differentiable from *C. diphtheriae* by the virulence test. It seems to be completely non-pathogenic for man and experimental animals.

A second species, *Corynebacterium xerosis*, has been isolated repeatedly from a form of conjunctivitis known as xerosis, but its etiologic relation to the disease is highly uncertain. It is also found on the skin where it is presumably a part of the normal bacterial flora, and it is probable that its presence in xerosis is that of a contaminant. It does not form a soluble toxin. *Corynebacterium acnes* has been found with some frequency in acne pustules but whether the association is causal is open to serious question. This organism stands somewhat apart from the other bacilli of the group in that it is microaerophilic and grows profusely under anaerobic conditions with the formation of a pink pigment. It also does not form a soluble toxin.

A number of species of corynebacteria are pathogenic for lower animals and rarely may infect man. *Corynebacterium progenes* is one of the commonest causes of purulent infections in cattle, sheep, pigs and goats; it is the cause of a form of mastitis, a few cases of abortion, arthritis and granulomatous

³⁷ For references and data see Snowman, *Epidemiological Info. Bull. (UNRRA)*, 1945, 1:157, and 1946, 2:147; Anderson, *Amer. Jour. Pub. Health*, 1947, 37:1.

cases, presumably because of their greater exposure to infection from contaminated water. Clinically the onset is sudden with rigors or chills, headache, pains in the back and limbs, often severe cramps in the calves, and sometimes nausea, vomiting, and diarrhoea. In a minority of the cases a macular eruption appears on the body and face on the 3rd or 4th day. Jaundice is uncommon. The disease lasts for 5-7 days, the temperature falling by lysis. There is sometimes a short relapse 1 or 2 days later. The case fatality is about 0.4 per cent. (Korthof 1932) The disease can be reproduced in human subjects by inoculation with pure cultures of the infecting organism. Leptospiræ can be demonstrated in the blood during the first 2 days of the disease. In practice bacteriological diagnosis is best made by examination of the blood serum for agglutinins, which often reach a titre of over 1/1000 by the 2nd week. The virulence of *Lepto. grippolyphosa* for guinea-pigs is very low or absent. The animal reservoir is constituted by field mice—*Microtus arvalis*, *Microtus agrestis*, *Apodemus sylvaticus*, and *Eutamias glareolus*.

The disease in man is not to be confused with the Swamp fever or Infectious Anæmia of horses, which is apparently due to a filtrable virus (see p. 2226).

Swineherds' Disease.—Clayton and Derrick (1937) described a 7-day fever in Queensland caused by an organism named *Lepto. pomona*. It affected mainly farmers and workers who were in contact with farm animals. The disease was mild, jaundice was rare, and death did not occur. Infection was found to be endemic in pigs and cattle. Later, Gsell (1916) described a disease among swineherds and butchers in Switzerland characterized by a double febrile peak, the second peak being accompanied by symptoms of serous meningitis. An organism cultivated from the blood of 7 of the patients on the 2nd to 5th day of illness proved to be identical with *Lepto. pomona*. Agglutinins were also present in the blood serum. Pigs in different parts of Switzerland were shown to be infected, no less than 59 per cent of pig sera contained specific agglutinins (see also Frey 1918).

A similar disease occurring among rural workers was described by Savino and Rennella (1911a) in the Argentine. The disease also occurs in northern Italy. According to Johnson (1950) gastro-intestinal and pulmonary symptoms are prominent, conjunctivitis is seen in nearly every case, arthralgia is common, and iritis is not infrequent. Schaeffer (1951) in the United States of America described an outbreak of an influenzal disease affecting 50 out of 80 young persons who had bathed in a creek in which dead pigs had been found; agglutinins to *L. pomona* were demonstrated in 18 out of 22 sera examined.

Seven-Day Fever of Queensland.—A disease practically identical with Swineherds' disease but caused by *Lepto. mutis* was recognized in Australia by Johnson (1912). Like *Lepto. pomona*, this organism was found to be a common parasite of pigs. The same disease was reported by Gsell and Wiesmann (1918) in Switzerland among young men who were in close contact with pigs. The fever was of 7-10 days' duration and was constantly accompanied by serous meningitis. Agglutinins to *Lepto. mutis* developed, though sometimes not till the 2nd or 3rd week.

Other Diseases caused by Leptospiræ.—Occasional cases of human disease have been reported caused by such organisms as *Lepto. ballum*, *Lepto. javanica*, and *Lepto. bangkinang*. Reference is made to them in Chapter 38.

lesions in bovines, and is associated with calf pneumonia as well. Infections of man have been reported.³⁸ It forms a soluble toxin, immunologically distinct from and considerably weaker than that of the diphtheria bacillus, which is hemolytic for rabbit erythrocytes, lethal for mice, and produces a dermal necrosis in the rabbit similar to that produced by diphtheria bacillus toxin. This bacterium and its toxin have been studied extensively by Lovell.³⁹

Corynebacterium ovis (*Corynebacterium pseudotuberculosis*) or the Preisz-Nocard bacillus is also a not uncommon pathogen of domestic animals. It produces a caseous lymphadenitis and ulcerative lymphangitis in sheep and horses referred to as pseudotuberculosis, and ulcerative lesions in other domestic animals. Like *C. pyogenes*, it forms a weak exotoxin distinct from diphtheria toxin. *Corynebacterium renale* is closely related serologically to *C. ovis* and produces purulent infections of the urinary tract in cattle, sheep, horses and

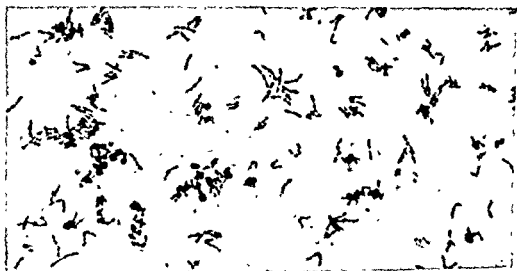


Fig. 144 *Corynebacterium pseudodiphtheriticum*; smear from pure culture stained with alkaline methylene blue. Note the irregular staining, club-shaped forms, and general close resemblance to *C. diphtheriae*. $\times 1050$.

dogs. *Corynebacterium equi* is the cause of a spontaneous pneumonia in foals and other infections in horses, this species is of interest in that it is variable in its reaction to the acid-fast stain, the coccoid forms retaining the stain while the bacillary forms take the counterstain, suggesting a relationship to the mycobacteria and acid-fast actinomycetes. *Corynebacterium enzymicum* has been isolated from man primarily, but has been found as the cause of an epidemic ophthalmia of sheep.⁴⁰ *Corynebacterium murisepticum* is the cause of a mouse septicemia and is apparently pathogenic for no other animals. The occurrence of these and other diphtheroid bacilli in diseases of domestic animals makes this group of microorganisms of considerable interest in veterinary medicine.

In addition to these a dozen or more authenticated species of corynebacteria are soil saprophytes or pathogenic for plants, producing diseases of wheat, alfalfa, ring rot of potato, bacterial canker of tomato and poinsettia, bean wilt, and the like.

³⁸ Cf. Ballard, Upsher and Seely: Amer. Jour. Clin. Path., 1947, 17:209.

³⁹ Lovell: Jour. Path. Bact., 1937, 45:339, *ibid.*, 1939, 49:329, *ibid.*, 1941, 52:295, *ibid.*, 1944, 56:525, Ver. Record, 1945, 57:386.

⁴⁰ Bruce: Canadian Jour. Comp. Med., 1943, 7:369.

disease; leptospiræ were isolated from the blood and milk during the fever, and demonstrated in the urine for several weeks afterwards. In Palestine the disease was recognized in 1937, where it attacks cattle, goats and sheep. According to Bernkopf, Olitzki and Stuczynski (1947), it ranges in severity from a subclinical infection to an acutely febrile and fatal form. Chronic cases with relapses are not uncommon. The disease can be transmitted serially in calves by intraperitoneal injection of blood taken during the pre-jaundiced stage. In animals that die interstitial nephritis and various degrees of liver damage are found. An organism isolated from the blood of an experimentally infected calf was named *Lepto. bovis*. Its exact identity is doubtful, but it appears to be the same as *Lepto. grippotyphosa*. The cattle are probably infected from voles—*Microtus guentheri* (van der Hoeden *et al.* 1953). In some parts of the world endemic infection of cattle occurs with *Lepto. pomona*, causing red water of calves, and with *Lepto. mitis*. Occasional cases of infection with *Lepto. icterohæmorrhagica* are on record (see Broom 1953).

Other Animals.—Numerous other animals suffer from leptospiral infection of one sort or another. In the Indonesian Republic cats are often infected with *Lepto. batavica* (Esseveld and Collier 1938) and occasionally with *Lepto. javanica* and *Lepto. semarang*. Mention has already been made of infection of goats and sheep with *Lepto. bovis*, which appears to be identical with *Lepto. grippotyphosa*. Infection of horses with *Lepto. icterohæmorrhagica*, *Lepto. canicola*, *Lepto. pomona*, and *Lepto. mitis* has been observed (see Savino and Rennella 1949); and the disease moon-blindness or periodic ophthalmia in horses is suspected of being of leptospiral origin, though the evidence is still inconclusive (see Heusser 1948, Joshua and Broom 1949). Dunkin and Laidlaw (1924-25) recorded the finding of *L. icterohæmorrhagica* in a jaundiced wild fox, and the same organism was found in apes (Wilbert and Delorme 1928). As will be evident from this chapter and from Chapter 38 leptospiral infections are very common in rodents, particularly rats and field mice. Bats, too, may be infected (see Chapter 38). For the animal reservoirs of different species of *Leptospira*, see van Thiel (1948), Savino and Rennella (1944b, 1949).

RAT-BITE FEVER

As has already been pointed out (p. 1471), there appear to be two distinct diseases known as rat-bite fever, one due to *Spirillum minus*, the other to an organism generally referred to as *Streptothrix moniliformis*, but better called *Actinobacillus muris*. We shall consider here only the spirillar type of infection.

Rat-bite fever, or *Sodoku* as it is called in Japan, is a disease that occasionally supervenes in man on the bite of a rat, or occasionally some other animal such as a cat (Mollaret and Bonnefoi 1938, Yamamoto 1939). The incubation period is generally 7 to 21 days, but it may extend to weeks or even months. The disease is ushered in by a sharp febrile paroxysm accompanied by swelling of the lymph glands and dark-red eruptions on the skin. Redness and swelling are noticed at the site of the wound, which during the incubation period has generally healed satisfactorily. There are often pains in the limbs on the affected side. After 3 or 4 days the attack comes to an end, but is succeeded by another in a few days. These febrile paroxysms with intermittent afebrile periods may be repeated for months, or even years. The fatality of the disease varies from about 2 to 10 per cent.

MYCOBACTERIUM

This genus includes a number of species of related bacteria which are most conveniently considered in three groups. The first includes the mammalian tubercle bacilli, *Mycobacterium tuberculosis* var. *hominis* and *Mycobacterium tuberculosis* var. *bovis*, and the avian tubercle bacillus, *Mycobacterium avium*. In the second group there are Hansen's bacillus or *Mycobacterium leprae*, and the rat leprosy bacillus, *Mycobacterium leprae murium*. The third group is made up of Johne's bacillus, or *Mycobacterium paratuberculosis*, and certain acid fast bacilli isolated from cold blooded animals, together with the saprophytic acid fast forms.

THE TUBERCLE BACILLI

Tuberculosis is an old disease of man and is still one of the most widespread, about 75,000 persons die of tuberculosis in the United States each year. Its infectious nature was suspected by Fracastorius in the early part of the sixteenth century, and Villemin showed, in 1865, that the disease could be transmitted by the inoculation of tuberculous material. It was in 1882 that Koch demonstrated the tubercle bacillus by special staining methods, isolated and grew it in pure culture, and reproduced the disease by the inoculation of the bacilli.

Morphology and Staining. The tubercle bacilli are slender, sometimes slightly curved rods 2 to 4 μ in length and 0.3 to 1.5 μ in breadth. They occur singly but are often found in small groups, sometimes in compact masses in which the individual bacilli cannot be distinguished. The bacilli of the human variety tend to be somewhat longer and more slender than those of the bovine type, but the morphology of both is variable and no distinction can be made on this basis. The bacillary form is generally retained in the tissues, in culture longer filamentous forms are sometimes seen together with swollen or club-shaped cells resembling the diphtheria bacillus. Branched forms are present in cultures of the avian tubercle bacillus but are rarely seen in cultures of the mammalian bacilli. The occurrence of filamentous forms and true branching indicates the close relation of these bacilli to the higher fungi, hence the name *Mycobacterium* and the placing of these microorganisms, together with the diphtheria bacillus, in the order Actinomycetales.

The tubercle bacillus is non-motile and non-spore forming and produces a capsular substance in artificial cultures, particularly when grown upon serum media. The granular structure of the individual cells is marked. Vacuoles often occur in abundance and may even give the stained cell the appearance of a chain of cocci. The significance of the small, deeply staining bodies sometimes observed within the cells is not clear, they do not exhibit the enhanced resistance characteristic of spores.

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The tubercle bacilli cannot be stained by the usual staining methods that are effective with other bacteria, for there is a marked resistance to the penetration of dyes into the cell that is associated with the presence of relatively large amounts of unsaponifiable wax. The cells may, however, be stained in two or three minutes by steaming carbol fuchsin or by prolonged (twenty-four to thirty-six hours) exposure to the dye at room temperature. Once stained, the bacilli are difficult to decolorize and resist the action of alcohol and dilute solutions of mineral acids and for that reason are termed "acid-fast." They may be demonstrated in smears by the Ziehl-Neelsen method, in which the smear is stained with hot carbol fuchsin, decolorized with acid alcohol, and counterstained with a dye of contrasting color. Methylene blue is most commonly used but some workers prefer other stains such as picric acid, Bismarck brown, etc. Non-acid-fast bacilli may be observed in young cultures.

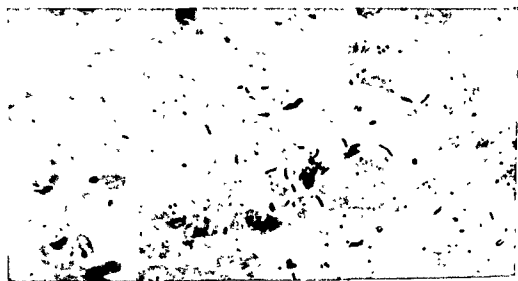


Fig. 145. *Mycobacterium tuberculosis*. Acid-fast stained smear of tuberculous sputum. $\times 1050$.

More recently fluorescent microscopy has been applied in the detection of tubercle bacilli in sputum smears, concentrates and similar material. The smear is stained with carbol-auramine, a solution of auramine in 3 per cent phenol, decolorized with acid alcohol, and examined in ultraviolet light. There is no counterstain. The ultraviolet irradiation need not be intense and a brilliant filament lamp with a blue ultraviolet-transmitting filter and an aluminum mirror suffice for use with the ordinary microscope.¹ The tubercle bacilli retain the dye which fluoresces in ultraviolet light, and appear as brilliant yellow bacilli on a dark background. The use of cresol in concentration of the bacilli interferes with the examination, for it fluoresces also. This staining method may also be used for tissue sections. Auramine is retained by the tubercle bacillus in the same way that fuchsin is retained, i.e., by virtue of the presence of the acid-fast wax mycolic acid, and bacilli rendered non-acid-fast by treatment with organic solvents no longer retain auramine.

¹ For detailed discussions of the method and its application see Richards, Kline and Leach: Amer. Rev. Tuberc., 1941, 44:255, Bogen *ibid*, 1941, 44:267, Lind and Shaughnessy: Jour. Lab. Clin. Med., 1942, 27:531.

CHAPTER 83

TYPHUS FEVER AND OTHER RICKETTSIAL DISEASES

THOUGH epidemic typhus has been known for centuries, and has played no small part in determining the course of history, it was not till 1909 that Nicolle and his colleagues (1911) first discovered that infection was spread by the body louse, and not till 1916 that da Rocha-Lima (see also 1951) demonstrated the ætiological agent of the disease, *Rickettsia prowazeki*. In 1907 Ricketts in the United States had succeeded in transmitting Rocky Mountain spotted fever to guinea-pigs, but the generic relationship of this disease to typhus fever remained obscure till Wolbach (1925) in 1916 demonstrated the rickettsial nature of the causative agent. During the war of 1914-18 a new fever, referred to as Trench fever, was met with; this was likewise found to be due to a species of *Rickettsia*. A sporadic form of typhus fever known as Brill's disease had been recognized for a long time in New York, a severer form known as *Tabardillo* in Mexico, and a milder form in Manchuria and the Far East. Broadly speaking, however, up till 1925, typhus fever was looked upon as one of the major epidemic diseases, which in certain areas might sometimes occur in a mild sporadic form. Rocky Mountain spotted fever and Trench fever were regarded as rickettsial diseases due to species of *Rickettsia* entirely distinct from that causing typhus fever.

In 1925 Fletcher and Lesslar (1925, 1926) found that the endemic typhus fever of the Federated Malay States could be divided serologically and epidemiologically into two different groups, one met with in the towns, the other occurring in country districts. Both differed in certain important respects from the classical type and from each other. These observations provided the main impetus for an extensive and detailed search in various parts of the world for other types of typhus fever. The result was to reveal the existence in several different countries of endemic typhus fever or typhus-like fevers many of which had previously been unrecognized.

We shall anticipate our discussion on the classification of the various rickettsial diseases of man by describing the diseases themselves, grouping them into six main categories: the typhus fever, spotted fever, rickettsialpox, tsutsugamushi, Q fever and trench fever groups.

THE TYPHUS FEVER GROUP

Two main varieties of typhus fever are known. The first is often referred to as the classical type; it is caused by *R. prowazeki*, and appears to be invariably louse-borne. The second is called the murine type; it is caused by *R. mooseri*,

The tubercle bacilli are gram-positive. Aniline gentian violet must be applied warm for two or three minutes. It has been suggested that the iodine solution plays no part in the retention of the stain as in the case of other gram positive bacteria and the failure to decolorize is a consequence of the acid fast nature of the cells.

Non-acid-fast but gram positive granules, known as *Much granules*, were described by Much in 1907 as occurring in the material from cold abscesses and elsewhere in which acid fast bacilli could not be demonstrated but which, nevertheless, proved to be infective. Considerable numbers of acid fast bacilli, perhaps 100,000 per milliliter, must be present, however, before there is a reasonable chance of finding them in smears. Much maintained that these granules are viable and virulent and give rise to typical acid fast rods. They have been observed by others but their significance is open to question. Some

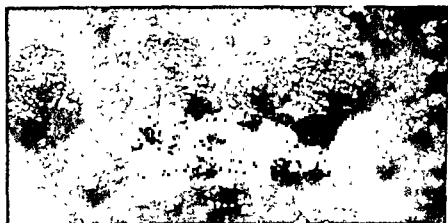


Fig. 146 Colonies of the human variety of the tubercle bacillus, H 37 strain, on Löwenstein's medium, five weeks' incubation $\times 3$

workers regard them as degeneration products or artifacts of the staining procedure.²

In broth cultures there is a thick, wrinkled skin of surface growth which tends to spread up the sides of the flask, masses of bacilli may become detached and sink to the bottom as a lumpy sediment. Growth on the surface of solid media is generally dry and granular with nodular, heaped up areas. The human variety of the tubercle bacillus usually produces a pale yellow or orange-yellow growth on serum-containing media and a creamy or white growth in the absence of serum. The bovine variety is not pigmented on serum media. Some avian strains give a faint pink-colored growth on egg media. A peculiar almond like odor is often noticeable in cultures of these bacteria.

Physiology. The tubercle bacillus is an aerobe and will not grow under completely anaerobic conditions. The mammalian varieties grow best at 37° C. and not at all below 30° C. or above 42° C., the optimum temperature for the avian type, however, is 40° C. Growth is relatively slow, and four to six weeks are generally required for an abundant growth, although minute colonies appear in eight to ten days. Most strains of the avian type adapt them-

² Cf. Potter and Yegorin, *Jour. Bact.*, 1945, 50:563.

fresh subjects by inhalation. There is reason to believe that the virus may remain alive for a year or more under these conditions, and the high infectivity of air-borne rickettsiæ has been amply demonstrated by laboratory observations (see Löffler and Mooser 1942, van den Ende *et al.* 1943). One attack of typhus fever confers a high degree of immunity, but immunity is not absolute. Second attacks do occur, mainly in the older age groups, and are usually mild (von Bormann 1950).

Bacteriology.

The causative agent of typhus fever is *Rickettsia prowazeki*, first described by da Rocha-Lima in 1916, and later in the same year by Töpfer (1916b). Da Rocha-Lima observed this organism in the lice taken from 95 per cent. of typhus patients; it was present in enormous numbers in the intestinal lumen and in the epithelial cells lining the gut wall (Fig. 298). The organisms stain a reddish-purple with



FIG. 298.—*Rickettsia prowazeki*, contained in an epithelial cell from the louse's mid-gut; both coccal and bacillary forms can be seen. Magnification 1,200 diameters (approx.). (After Wolbach, Todd and Palfrey.)

Giemsa, are very small, of short elliptic or olive-shape, often in pairs, and are surrounded by a paler staining substance; occasional very short, and very long forms up to 1.5–2 μ , may be seen. They were never found in lice fed on normal persons, or on typhus convalescents, but appeared regularly, and in enormous numbers, in lice fed on typhus patients. The time elapsing between feeding and the appearance of rickettsiæ in the gut depended on the temperature at which the lice were kept; at 23° C. no rickettsiæ developed at all, but at 32° C. they appeared in about 5 to 8 days, and persisted indefinitely. At about the same time as the rickettsiæ developed, the lice became infective for guinea-pigs.

It should be noted that not every louse fed on a typhus patient develops rickettsiæ or becomes infective. Wolbach, Todd and Palfrey (1922), for example, working in Poland in 1920, fed batches of 30 to 40 lice on typhus patients for an average period of 12 to 16 days, and found that, out of 52 experiments, in only 27 did the lice develop rickettsiæ. In a later series of experiments they also found that only a similar proportion of lice became infective for guinea-pigs. The actual proportion observed was very low, and is probably accounted for by imperfect technique in dealing with the lice, during

the latter part of their work the proportion of positive results was considerably higher. Arkwright, Bacot and Duncan (1919) similarly concluded that rickettsiæ do not appear regularly in the excreta of lice fed on typhus patients. Whether this is due to variation in the lice, or, as seems more probable, to the relatively low infectivity of typhus blood, is not known. The significant point is the general parallelism that exists between the presence of rickettsiæ in the louse, and the infectivity of the louse for guinea-pigs.

R. prowazeki was cultivated by Wolbach and Schlesinger (1923–24) in tissue cultures. Small pieces of the brain of typhus guinea-pigs were seeded into normal guinea-pig plasma, and incubated in the usual way over hollow-ground slides. Rickettsiæ were found in the endothelial cells of the vessel walls; primary cultures remained virulent for 8 to 15 days, second generation cultures up to 28 days. The rickettsiæ showed signs of multiplication within the cells, they appeared as coccoid, bacillary, and filamentous forms similar to those found in lice.

selves readily to culture on artificial media and in time are able to grow much more rapidly, but others remain slow-growing.

The tubercle bacillus is difficult to cultivate upon primary isolation. Blood serum, coagulated by inspissation, was used by Koch, and this medium still remains one of the most satisfactory. Media containing eggs, either the yolk or yolk and white combined, glycerol, and sometimes dyes, are also used for primary isolation. Dorset's egg medium is simply whole egg mixed with a little water and coagulated in slant form by inspissation. Petroff's medium, one which is widely used, consists of an infusion base to which eggs, glycerol and gentian violet have been added, and is sterilized by inspissation. Lowenstein's medium is more complex and contains egg, potato meal, bone marrow infusion, citrate, glycerol and asparagin. Glycerolated potato is used by the French workers. Corper's medium is glycerolated potato with the modification that the pieces of potato are soaked for a short time in a solution of crystal violet before sterilization with the glycerol solution. The dye in this and Petroff's medium serves to inhibit the growth of contaminating bacteria.

The human variety of the tubercle bacillus grows more abundantly on all of these media than does the bovine variety, and for that reason it is termed "eugonic" and the bovine type "dysgonic." These two varieties also differ in that glycerol is markedly favorable to the growth of the human type but does not so affect the bovine type. It is not known why glycerol exerts this favorable effect; attempts to substitute related compounds such as isopropyl alcohol, propyl alcohol, glycol, trimethylene glycol and inositol have not been successful. Glucose, however, acts in much the same manner as glycerol. Egg-yolk has been reported to contain a lipid growth factor, but this appears to stimulate rather than be essential to growth. Thiamine, pyridoxine and riboflavin do not stimulate growth.

Growth occurs much more readily and upon simpler media after primary isolation. The human tubercle bacillus grows well upon nutrient agar or broth containing glycerol (2 to 5 per cent) and has been cultivated in a variety of synthetic solutions. One of the best known of these is Long's synthetic medium which contains glycerol, asparagin, citrate and inorganic salts. Dubos and his co-workers³ have found that growth is facilitated and occurs diffusely throughout the liquid medium in the presence of certain water-soluble lipids. A medium containing asparagin, glucose, phosphate, citrate, bovine serum albumin, magnesium sulfate and a lipid commercially designated Tween 80 (a polyoxyethylene derivative of sorbitan mono-oleate), will give visible growth of the human variety of tubercle bacillus within two weeks. The medium becomes inhibitory in time owing to the activity of lipase contaminating bovine albumin preparations, which liberates oleic acid to bacteriostatic concentrations from the Tween 80, the effect can be eliminated by the use of commercial crystalline bovine serum albumin, the addition of 0.01 per cent sodium fluoride, etc. The bovine type grows poorly or not at all on these media and is benefited only slightly by the presence of glycerol. The avian type of tubercle bacillus, however, grows better than the human type after a few transfers and good growth may be obtained on nutrient agar in the absence of glycerol. Like the human type, however, the avian bacillus grows much more profusely in the

³ Dubos: Proc. Soc. Exp. Biol. Med., 1945, 58:361; Dubos and Davis Jour. Exp. Med., 1946, 83:409; Dubos and Middlebrook. Amer. Rev. Tuberc., 1947, 56:334.

rôle of *R. prowazeki* in typhus, and there is no longer any reason to doubt the part played by this organism in the causation of the disease. (For reviews of typhus fever, see Clavero and Gallardo 1941, Gordon 1948, Zinsser 1948, Snyder 1948)

Murine Typhus Fever.

The recognition of an endemic form of typhus fever in the United States, occurring sporadically in man, and spreading not by the louse but by the rat flea *Xenopsylla cheopis*, was due to the brilliant work of a group of American and Mexican workers, prominent among whom were Maxcy, Mooser and Dyer (see Maxcy 1926, 1928, 1929, Mooser 1928, Mooser *et al.* 1931, Dyer *et al.* 1931a, b, c, d, 1932a, b, c, Ceder *et al.* 1931, Rumreich 1933). The disease is endemic, occurs in the summer and autumn, is commonest in seaports along the Atlantic border, is not associated with overcrowding, has no predilection for the lower classes, is non-contagious, and has a case fatality of only 1 per cent. It is caused by a virus, often referred to as *R. mooseri*, which differs in some respects from *R. prowazeki*, and which is responsible for causing an extremely mild endemic infection of rats. Sporadic cases in man are liable to occur in persons brought into close contact with these animals, infection being transferred by the rat flea *X. cheopis*. The virus is passed from rat to rat by the rat louse, *Polyplax spinulosus*, and by rat fleas. The rats are never very sick, and the fleas may harbour the virus for over a month and recover—both circumstances indicating a well-established and possibly ancient parasitism (Zinsser 1937). On the other hand, there seems very little doubt that, under suitable conditions, the disease may become epidemic in man and be spread by the body louse. This is apparently what has happened in Mexico where, in addition to sporadic cases of flea-borne typhus, an epidemic disease known as *Tabardillo* occurs. Like classical typhus, it has its maximum prevalence in the winter, is associated with overcrowding, is most prevalent in the lower classes of the population, is contagious, is carried by lice, and has a fairly high case-fatality rate.

The demonstration by American workers of the part played by the rat and the rat flea in the spread of endemic typhus was soon followed by the recognition of the disease in other parts of the world. It is called by various local names, such as Toulon typhus, endemic typhus of Moscow, Manchurian typhus, shop typhus of Malaya, and so on. Unlike the classical form, which is commonest in temperate climates, the endemic murine form of typhus tends to be more prevalent in warm countries. Examination of wild rats in numerous areas where the disease is endemic has shown that these animals often give a positive Weil-Felix reaction to *Proteus* OX 19, and that they are latently infected with a murine typhus virus (Penfold and Corkill 1928, Marcandier *et al.* 1931, Kodama and Takahashi 1931, Kodama *et al.* 1932a, b, Brumpt 1932b, Netter 1932, Lépine 1933, Lawton and Murray 1933, Chao-Jen Wu 1933, Kritschewski and Solowiow 1931, Suzuki 1931, Nicolle and Sparrow 1931, Epstein and Silvers 1931, Zinsser 1937, Wolbach 1911; see also Tonking 1932-33, Rickard and Riley 1918, Freeman *et al.* 1919).

Comparison of the Classical and the Murine Typhus Viruses.—The two viruses of typhus fever, *R. prowazeki* and *R. mooseri*, are very closely related. Both stimulate the production of agglutinins acting on *Proteus* OX 19. Cross-immunity studies have often failed to elicit any difference between them (Zinsser and Castaneda 1930a, b, 1932, 1934, Zinsser and Batchelder 1930, Mooser and Dummer 1930, Weigl and Hertzog 1933, Mooser *et al.* 1934, Finlayson and Grobler 1940a, b);

presence of glycerol. These cultural differences in the three types of tubercle bacilli are of little significance in their early differentiation, for several transfers over a period of months are necessary before they are obvious. They are summarized in the table on p. 642.

The biochemical reactions of the tubercle bacilli have not been studied at length. Growth occurs in milk, but no visible change is produced. Indol is said not to be formed. No acidity is developed in sugar broths, but it has been found by analytical methods that dextrose, arabinose and sometimes sucrose are utilized while lactose is not. Whether the oxidation proceeds to completion or whether formed acids are neutralized by liberated ammonia is not known. In glycerol broth, cultures of the bovine type become alkaline while those of the human type become slightly acid. It has been reported that the source of alkali is the nitrogenous constituents of the medium, and acid is produced from glycerol but is further oxidized. All intermediate gradations occur, however, and no differentiation between the two types is possible on this basis.

Chemical Composition. The chemical composition of the tubercle bacilli has been more intensively investigated than that of any other bacteria. These bacilli are of particular interest in this connection because of their high content of lipoidal substances which may make up as much as 40 per cent of the dry weight. Protein, a considerable proportion of which is nucleoprotein, makes up about half the dry weight and polysaccharides are found in relatively small amount.⁴

The lipids have been studied at length by Anderson⁵ and his co-workers. In addition to neutral fat, two general types of material may be distinguished.

(a) Phospholipid, containing saturated and unsaturated fatty acids including the well-known palmitic, linoleic and linolenic acids, together with two acids peculiar to the tubercle bacillus, *phthioic acid*, isomeric with cerotic acid, and *tuberculostearic acid*, isomeric with stearic acid and optically inactive.

(b) An acid fast wax, containing polysaccharides hydrolyzing to mannose, arabinose and galactose, a soft wax which is a complex glyceride, and an unsaponifiable wax (acid fast) made up of higher alcohols and including a high molecular weight saturated hydroxymethoxy acid termed *mycolic acid*, a higher alcohol designated *phthiocerol*, and a levorotatory fatty acid, *mycocerotic acid*.

Some of these substances appear to be physiologically active. The unsaponifiable acid fast wax apparently stimulates the multiplication of undifferentiated connective tissue cells, and *phthioic acid* induces a proliferation of epithelioid cells.⁶ Whether they are immunologically active is not clear. It may be noted that a yellow pigment is found in the neutral fat which is designated *phthiocol* and is a hydroxynaphthaquinone which may be reversibly reduced at a relatively low potential.

Polysaccharide mixtures, containing immunologically active and inactive substances, have been isolated from mammalian tubercle bacilli⁷ but their significance is not as yet understood. The protein constituents of the cell appear

⁴ See the review of the protein constituents of the tubercle bacillus by Seibert. *Bact. Rev.*, 1941, 5:69.

⁵ Cf. Anderson. *Physiol. Rev.*, 1932, 12:166; *Chem. Rev.*, 1941, 29:225.

⁶ Cf. Sabin. *Physiol. Rev.*, 1932, 12:141; *Amer. Rev. Tuberc.*, 1941, 44:415.

⁷ See Menzel and Heidelberger. *Jour. Biol. Chem.*, 1939, 127:221.

Rocky Mountain Spotted Fever.

This disease, which has been known for many years, occurs in North America. Two types used to be recognized: a Western type with a case fatality of 70 per cent. or so; and an Eastern type occurring nearer the Atlantic border with a case fatality of only 20-25 per cent. (Badger 1933a). Recent workers, however, have cast doubt on this difference and tend to regard them both as one disease (Parker 1938, Wolbach 1941). The difference in the case-fatality rate can be explained largely by the age distribution of the two diseases, the Western form being commoner in older, the Eastern in younger persons. Rocky Mountain spotted fever affects those engaged in outdoor pursuits. It has a striking seasonal incidence, being commonest during the summer and practically absent during the winter months (Hampton and Eubank 1938). The infecting agent, *Rickettsia rickettsi* (*Dermacentrozetes rickettsi*), is carried by ticks. The Rocky Mountain wood tick, *Dermacentor andersoni* Stiles, seems to be mainly concerned in the west and the American dog tick, *Dermacentor variabilis*, in the east (Dyer *et al.* 1931e). Some cases—usually mild—appear to result from the bite of the rabbit tick *Hæmaphysalis leporis-palustris*, which is very widespread (see Parker 1938); and there is evidence that others may follow the bite of the Lone Star tick, *Amblyomma americanum*—so called from the star-shaped marking on its back—which occurs on dogs and cats, and which bites man in all stages of its development from the larva to the adult (Parker *et al.* 1943). The severity of the disease varies greatly. In mild cases the incubation period ranges from 3 to 14 days, in severe cases from 2 to 5 days. The fever lasts as a rule for 2 to 3 weeks. Convalescence is prolonged.

Ricketts (1907) was successful in transmitting infection to guinea-pigs and monkeys. Besides showing a febrile reaction, inoculated guinea-pigs often develop swellings and hæmorrhages of the scrotum and ears, which may go on to necrosis; the spleen is considerably enlarged (see Munter 1928). The Western type often proves highly fatal to guinea-pigs. Rabbits also develop a febrile disease and sometimes a scrotal reaction; introduction of the virus into the anterior chamber of the eye is less often followed by an acute reaction than occurs with the scrub typhus or tsutsugamushi virus (Lewthwaite and Savor 1936b). Rats and mice can be infected, but the disease pursues a symptomless course (Fukuda 1929).

In human and animal lesions the organisms are found with comparative ease. The lesions themselves resemble those of typhus, but according to Lillie (1931, 1941), endovascular proliferation, degeneration, and thrombosis tend to be commoner. Moreover, in Rocky Mountain spotted fever the medial coat of the blood vessels is said to be affected as well as the endothelial lining (see Wolbach 1941). The blood vessels of the skin and reproductive organs suffer most severely.

The organism can be grown in tissue culture, and is distinguished from *R. prowazeki* by its multiplication in the nuclei of infected cells, where it forms compact spherical colonies (Pinkerton and Hass 1932). The typhus virus multiplies only in the cytoplasm. In the tick the organism presents three forms: (1) an intracellular bacillus-like form, without chromatoid granules, present only in the cytoplasm of the cells of the alimentary tract, (2) a small, rod-shaped form with chromatoid granules, present within the cells and the nuclei of many tissues, (3) a larger lanceolate diploid form, which persists in the tissues, notably the salivary glands, long after the other forms have disappeared; this lanceolate form is characterized by its chromatoid staining reaction; it is the only form found in the eggs of infected ticks (see Wolbach 1925). It may be noted that ticks, once

to be the most important immunologically and have been studied in connection with the preparation and activity of the various tuberculins which are considered in a later section.

Resistance. Although exhibiting much the same degree of resistance to heat as the vegetative cells of other bacteria, the tubercle bacilli are relatively highly resistant to drying, chemical disinfectants and other deleterious environmental influences, very likely as a consequence of their content of wax. In putrefying sputum the bacilli may remain viable for weeks or months and in dried sputum kept in a cool dark place for as long as six to eight months. Sputum that is completely dried, so that particles are capable of floating as dust in the air, may be infective for eight to ten days.⁸ In dried sputum they may survive 100° C. for an hour but are killed in the usual way by moist heat. Phenol penetrates the bacilli only slowly and a 5 per cent solution requires twenty-four hours to kill the bacilli in sputum. The action of other disinfectants is similarly retarded, and it may be noted that the hypochlorites have almost no effect on these bacteria. Tubercle bacilli, however, are readily killed by exposure to direct sunlight; bacilli from cultures are killed within two hours but in sputum may survive twenty to thirty hours of such direct exposure.

Variation. The variability of the tubercle bacilli has been studied extensively, particularly in recent years, but with inconclusive results. Colonial variants, thought by some to be analogous to the S and R variants of other bacteria, have been observed, and it has been claimed by some workers that the S type is the more virulent, and by others that virulence and colonial morphology are independent. It may be noted that the colonial morphology of the tubercle bacilli is, to a considerable degree, a transient adaptation to environmental conditions and prompt alteration of colonial appearance results on transfer to a different medium. For example, Steenken⁹ has observed that colonies growing in the presence of ether extract of egg yolk are smooth and markedly different from the usual colonial type, but the effect is only a temporary physical one. The status of the S-R variation in the tubercle bacilli is, then, by no means clear as yet.

Bacille Calmette-Guérin. A bovine strain of the tubercle bacillus was rendered completely avirulent by Calmette,¹⁰ who cultivated it over a long period of time (230 transfers in thirteen years) on bile-glycerol-potato medium. This strain is designated as BCG (*Bacille Calmette-Guérin*) and has been of particular interest in connection with active immunization against tuberculosis. The nature of the change which resulted in the loss of virulence is completely unknown. The loss appears to be permanent and virulence does not reappear on transfer to ordinary media, Petroff,¹¹ however, has separated "rough" and "smooth" variants of BCG, and the "smooth" type proved virulent for guinea pigs.

Life Cycles. The pleomorphic tendencies of the tubercle bacilli, coupled

⁸ For example, see Smith: *Amer. Rev. Tuberc.*, 1942, 45:334

⁹ Steenken *Amer. Rev. Tuberc.*, 1940, 42:422.

¹⁰ Cf. Calmette. *Tubercle Bacillus Infection and Tuberculosis in Man and Animals* (transl.). Williams & Wilkins Company, Baltimore. 1923.

¹¹ Petroff *Proc. Soc. Exp. Biol. Med.*, 1927, 24:632, Petroff, Branch and Steenken: *ibid.*, 1927, 25:14.

different ticks—*Amblyomma hebraeum*, *Rhipicephalus appendiculatus*, and *Boophilus decoloratus*—and by all stages of the dog tick, *Hæmaphysalis leachi* (see Gear 1941). The virus gives rise to a mild fever in guinea-pigs, with little or no scrotal reaction. It can apparently be cultivated on the chorio-allantoic membrane of the developing chick embryo (Alexander, Mason and Neitz 1939). According to Pijper (1936) no cross-immunity exists between tick-bite fever and fièvre boutonneuse, and according to Pijper and Crocker (1938) there appears to be little relation between tick-bite fever and Rocky Mountain spotted fever. Both of these conclusions, however, are disputed by Mason and Alexander (1939*a, b*), who regard tick-bite fever as a member of the spotted fever group, and suggest that the virus should be given the varietal name of *pijperi*.

North Queensland Tick Typhus.

This disease, which was described by Andrew, Bonnin and Williams (1946), resembles a mild form of Rocky Mountain spotted fever. It is characterized by an eschar at the site of the tick bite, regional adenitis, general adenopathy, fever lasting as a rule about a week, a rash appearing on the 3rd or 4th day of the fever, and the development of agglutinins to *Proteus* OX 19, less often to OX 2. Mice and guinea-pigs are susceptible. In guinea-pigs injected intraperitoneally there is a hæmorrhagic scrotal reaction. The agent can be grown in the yolk sac of the developing chick embryo, and in tissue culture. Growth occurs in the nuclei as well as in the cytoplasm (Funder and Jackson 1946). Rabbits injected with tunica suspension from infected guinea-pigs develop complement-fixing bodies to *R. rickettsi*, but both by complement-fixation and by cross-protection tests in guinea-pigs the organism appears to differ from any of the known members of the spotted fever group (Plotz *et al.* 1946, Lackman and Parker 1948).

THE RICKETTSIALPOX GROUP

Rickettsialpox was the name given by Huebner, Stamps and Armstrong (1946) to a febrile disease resembling chicken-pox occurring in epidemic form on a large housing estate in the borough of Queens, New York City. The disease was recognized in other parts of New York City (Greenberg *et al.* 1947), and later in Boston (Fuller *et al.* 1951). One probable case was recorded in French Equatorial Africa (Le Gac and Giroud 1951). The causative organism, *R. akari*, is carried by a mite, *Allodermanyssus sanguineus*, that infests house mice (*Mus musculus*). Clinically, a papulo-vesicular lesion occurs at the site of the bite, accompanied by enlargement of the regional lymphatic nodes. Three to ten days later a remittent fever develops, lasting for less than a week. Within a day or two of its onset, a generalized papulo-vesicular rash appears, and persists until shortly after defervescence. The initial local lesion remains as a black eschar for several weeks. The disease is not fatal, and for this reason little is known about its pathology. During the fever the organism can be isolated from the patient's blood by intraperitoneal injection of mice. Passage from mouse to mouse is possible by inoculation of liver, spleen or brain. The organism can be cultivated in the yolk sac of the developing chick embryo. Complement-fixing bodies are present in the serum of convalescent patients. The organism may also be isolated from naturally infected house mice (Huebner *et al.* 1947), and complement-fixing bodies may be demonstrated in their

with the occurrence of non acid fast rods in young cultures and the granular elements described by Much, have been interpreted by a number of workers as indicative of a cyclic succession of morphologic types, or life cycle, through which these bacilli go. As pointed out in an earlier chapter (Chap. 6), the evidence for such developmental cycles is not definitive but rather a matter of interpretation. Whether swollen, misshapen cells, filamentous or branching forms and granular elements, such as those described by Much, are to be regarded as sexually reproducing forms, gonidia and the like, or as products of degenerative changes is as yet purely a matter of opinion. It may be noted that the objective evidence of micromotion pictures does not support the assumption of a complex life cycle in the development of the tubercle bacilli.¹²

Filterable Forms Associated by some with the concept of life cycles is that of the existence of tubercle bacilli in a filterable form or "ultravirus." There seems to be little doubt that some cultures or preparations of tubercle bacilli contain forms that may pass diatomaceous earth or unglazed porcelain filters, but whether these are dwarfed bacilli or fragments of bacilli with the power to regenerate, or whether they represent a distinct "phase" in the life history of the microorganisms has yet to be determined.

Pathogenicity for Man. In the United States in 1945 there were 106,716 cases of all forms of tuberculosis, and of these 65,843 were cases of pulmonary tuberculosis. Tuberculosis is among the first three leading causes of death in a relatively large portion of the life span (15 to 49) and holds first place in the 15-34 age group, second in the 35-39 age group, and third in the 40-49 group. In 1939-41 it accounted for 10.4 per cent of the white male, 22.9 per cent of the white female, 28.8 per cent of the non-white male and 37.8 per cent of the non-white female deaths.¹³ Tuberculosis is clearly one of the great killing diseases of mankind.

The mammalian tubercle bacilli, both bovine and human varieties, are pathogenic for man. The human type is practically always responsible for pulmonary tuberculosis in adults and is usually found in children also. The bovine variety may occur occasionally in pulmonary tuberculosis in children but is more often found in infections of other tissues. More than half of the cases of cervical adenitis and abdominal tuberculosis in children are infections with the bovine tubercle bacillus, and it has been estimated (1910) that this type was responsible for 6 to 10 per cent of the deaths from tuberculosis in young children; the percentage is probably lower now as a result of decreased incidence of tuberculosis in cattle in this country. Bovine tuberculosis is, then, a slight, possibly a negligible, factor in adults but is a serious matter in children under five years of age. Mixed infections with the two types of tubercle bacilli have been reported but are rare.

The avian tubercle bacillus is, for all practical purposes, non pathogenic for man, although a few instances of human infection, including pulmonary tuberculosis, have been reported. It has been supposed by some that Hodgkin's disease—a granulomatous inflammation of the lymphadenoid tissues of the body—is an infection with avian tubercle bacilli, but this appears not to be true.

Routes of Infection. The tubercle bacillus may enter the body by way of

¹² Wicks, *Amer. Rev. Tuberc.*, 1934, 29:359.

¹³ Yerushalmy, Hillelsoe and Palmer. *Pub. Health Rep.*, 1943, 58:1457.

field rat, *Mus diardii* (see Zinsser 1937), appear to constitute a reservoir of infection for the mite vector, *Trombicula deliensis*.

The viruses of these three diseases all closely resemble each other in being comparatively non-infective for guinea-pigs, in producing an inapparent infection in white rats, in causing a severe reaction in the rabbit's eye after intra-ocular injection, and in giving rise to a local necrotic lesion accompanied by fever on intradermal inoculation into the monkey. The serum of convalescent monkeys and rabbits agglutinates *Proteus OX K* but not *OX 19*. The serum of convalescent patients behaves similarly, though the serum of tsutsugamushi patients is said to agglutinate *Proteus OX K* to a lower titre than that of scrub fever or mite fever patients (Wolff 1931a, Kawamura *et al.* 1935). Cross-immunity experiments in the rabbit indicate the very close relationship of the three viruses to each other (see Nagayo *et al.* 1930, Kawamura and Imagawa 1931, Wolff 1931b, Kouwenaar and Wolff 1933, 1934, 1935, 1936, Wolff and Kouwenaar 1933, Lewthwaite and Savor 1931, 1936a, b). Kouwenaar and Wolff (1936) are of the opinion that mite fever and tsutsugamushi can be distinguished by monkey inoculation. The observations, however, of Lewthwaite and Savor (1936d, 1940a) seem to leave little doubt that there is complete cross-immunity between the three diseases. Some of the discrepant observations can probably be explained by the antigenic heterogeneity of *R nipponica*. If we accept the essential identity of these three diseases, we must follow Lewthwaite and Savor (1910b) in giving up the terms "scrub typhus" and "mite typhus" in favour of the general term tsutsugamushi (dangerous or disease mites).

During the 2nd world war tsutsugamushi was widespread among the Forces in the Far East. Three major explosive outbreaks occurred when troops occupied heavily infested ground. The case-fatality rate varied greatly, ranging from 0.6 per cent in the Owi-Biah outbreak to 35.3 per cent. in one of the smaller outbreaks on Goodenough Island (Philip 1918). The disease has a wider distribution than was originally thought. It occurs in practically all parts of India, in Burma, Indo-China, Malaya, Sumatra, Borneo, Java, New Guinea, the Solomon Islands, Formosa, and parts of China and Japan. Cases are seen throughout the year, but in Assam and Burma they tend to be concentrated about the beginning and end of the monsoon. Both *Trombicula akamushi* (akamushi = red mite) and *Trombicula deliensis* are known vectors, the latter being the more widely distributed. Proof of the part played by *T. deliensis* in the spread of the disease was brought by Mackie and his colleagues (1916) and by Krishnan and his colleagues (1949), who not only isolated *R nipponica* from the mites in nature, but by rearing the larvæ in the laboratory were able to demonstrate the transovarian passage of the infection.

Observations on experimentally infected human volunteers showed that the incubation period of the disease is 8-10 days, that immunity to infection with the homologous strains of *R nipponica* is fairly lasting, but that persons convalescent from infection with one type may be infected after a short time with a strain belonging to a heterologous type (Kawamura *et al.* 1939, Smadel, Ley *et al.* 1950). For further information on the bacteriology and pathology of tsutsugamushi the reader is referred to papers by Ogata (1931, 1935), Nagayo *et al.* (1931), Lewthwaite

the genito-urinary tract, the conjunctiva, the skin, the alimentary tract and the respiratory tract. Primary infection of the genito-urinary tract is possible but, under natural conditions, rarely occurs. Infection through the conjunctiva takes place readily under experimental conditions; its frequency under natural conditions is not known, for the cervical lymph glands, where the infection would first appear, are readily infected by other channels. Infection through the skin is relatively rare; whether the bacilli can penetrate the intact skin is uncertain, but they may enter through abrasions or other traumatic injuries. Primary infection of the skin generally results in verruca tuberculosa (pathologist's wart) or lupus vulgaris.

Primary infection by the alimentary tract is a consequence of the ingestion of tubercle bacilli in infected food, most commonly milk, and occurs with con-

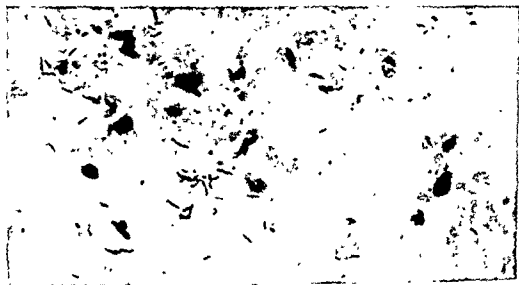


Fig. 147. Tubercle bacillus. Acid-fast stained smear of pus from a liver abscess in a rhesus monkey. $\times 1050$.

siderable frequency in children. Secondary infection may occur, especially in children, by swallowing tuberculous material of respiratory origin. In the upper portion of the alimentary tract the bacilli enter the body tissues through the faucial, pharyngeal and lingual lymphoid follicles and first affect the upper cervical and retro-pharyngeal lymph glands. Tuberculous lesions of the tonsils, it may be noted, are not infrequent though rarely conspicuous. The stomach is rarely a portal of entry, but the bacilli penetrate the intestinal mucosa by way of Peyer's patches.

The respiratory tract is the most frequent and most important route of infection with the tubercle bacillus. The coarser infectious particles in inspired air are filtered out and deposited on the nasal, buccal and pharyngeal surfaces and the bacilli, upon penetration, set up focal infections in the local lymphatic tissue. Fine droplets or particles of dust, however, may, and frequently do, enter the lungs directly.

The Spread of Infection in the Body. Tubercle bacilli are disseminated through the body from primary or secondary foci of infection by way of the lymph, the blood stream, or directly by extension along contiguous surfaces.

holocyclus, transferred infection from bandicoots to cattle, and thus led to infection of the cattle tick, *Boophilus annulatus*; that the cattle tick excreted the rickettsiae in its faeces; and that the dried cattle tick faeces were inhaled by man, so explaining the frequent occurrence of pneumonitis as a primary symptom of the illness (Burnet 1942). (For a review of the disease in Queensland, see Derrick 1944.)

The discovery of the disease in the United States was due to Davis and Cox (1938) who, in 1935, demonstrated a filter-passing agent in wood ticks, *Dermacentor andersoni*, collected near Nine Mile Creek, Montana. Parker and Davis (1938) described its experimental transmission by the tick. Dyer (1938) reported the occurrence of infection in a laboratory worker, and later (1939) pointed out the close relationship of this disease to the Q fever of Australia. The American virus is said to be more virulent for laboratory animals than the Australian virus, but there appears to be complete cross-immunity between the two diseases (Dyer 1939).

During the 2nd world war the disease occurred fairly extensively in the Mediterranean area. At least eight outbreaks resembling virus pneumonia were recognized among allied troops in Italy, Greece and Corsica during the winter and spring of 1944-5 (Robbins *et al.* 1946, Caughey and Dudgeon 1947). The incubation period was estimated at 19-20 days, with a possible range of 14-26 days. The mode of infection was not ascertained with certainty, but some of the outbreaks were explosive and seemed to be associated with the inhalation of dust from hay or straw. The disease was studied in Greece by Caminopetros (1948), who found that both sheep and goats suffered from the infection, and that they excreted the organism in their milk during the whole of the lactation period.

After the war outbreaks of the disease in the United States were recognized among stockhandlers and slaughterhouse workers in Texas (Topping *et al.* 1947) and Chicago (Shepard 1947). Later Q fever was found to be widely endemic in California. Epidemiological investigations showed that in northern California the disease was associated with sheep and goats, and in southern California with cattle, which were found to excrete the organism in the milk (Shepard and Huebner 1948, Huebner *et al.* 1948, Lennette *et al.* 1949, Bell *et al.* 1950). Judging by the frequency of complement-fixing antibodies in the blood, Bell, Beck and Huebner (1950) estimated that probably over 50,000 persons in southern California had suffered from Q fever during the preceding few years. In south California the incidence is highest among dairy workers, stockworkers, and those drinking raw milk. Undoubtedly man often becomes infected through drinking raw milk, but another source appears to be the parturient cow whose placenta contains enormous numbers of rickettsiae (Luoto and Huebner 1950). In north California most of the cases occur among males and most, but by no means all, have a history of contact with domestic livestock. The inhalation of infected dust, in which rickettsiae have been demonstrated (DeLay *et al.* 1950), is supposed to be the common mode of infection. In Great Britain infection of human beings is mainly derived from cattle, though sheep may play a small part (Marmion *et al.* 1953, 1954).

The disease has now been recognized in several parts of the world, including many countries of Europe such as Great Britain (MacCallum *et al.* 1949, Marmion *et al.* 1953, see also Slavin 1952), Switzerland (Gsell 1948), Germany (Henri and Germer 1948), Yugoslavia (Murray *et al.* 1951), Portugal (Fonseca *et al.* 1949, 1951), France, Spain, and Turkey, and in South America. Numerous outbreaks have occurred among laboratory workers (Robbins *et al.* 1946, Huebner 1947, Derrick 1953).

Distribution via the lymphatics occurs more readily in children than in adults, and the bacilli may localize at almost any point but most commonly in the lymph nodes. Bacilli present in the thoracic duct may, of course, gain entrance to the blood stream. The blood stream may also be invaded directly through erosion of a vessel wall by a focus of infection. The bacilli are transported throughout the body by the blood and give rise to acute miliary or chronic disseminated tuberculosis. Spread by extension occurs most frequently in ulcerative pulmonary tuberculosis with the breaking down of foci of infection and the consequent presence of bacilli in the sputum. The pleura and pericardium may be invaded directly from a focus of infection in the lungs. The infection of such serous surfaces may be localized and fibrinous and result in adhesions, or may assume an acute miliary form. Direct extension may also occur elsewhere, as from the kidneys to the ureters and bladder, or into the peritoneal cavity and adjacent areas from intestinal ulcers.

It will be clear that practically every organ and tissue of the body may be invaded by the tubercle bacillus. Not all are invaded with equal frequency, however. Tuberculosis in man is most commonly the pulmonary form with a primary invasion of the apices, and over 90 per cent of the deaths from tuberculosis are due to the pulmonary type. Whether primary infection is a direct consequence of inhalation as believed by Koch, or whether it is in most cases of hematogenous origin following a preliminary infection of the lymphatic system as urged by Calmette, is not altogether clear, but on the whole the evidence favors inhalation infection. Although pulmonary tuberculosis is by far the most common form of infection in adults, it is somewhat less frequent in children, but precisely how much less is not known with certainty. Pulmonary infection in children differs from that in adults in that the hilum glands are involved. Involvement of the lymphatics is frequent in children. The anatomical distribution of the lesions usually takes one or the other of two well defined forms, in the one the lesions are found predominantly in the tracheo-bronchial lymphatics, and in the other in the mesenteric lymph nodes. In general, children show a tendency toward generalized infection. In 1945 92 per cent of deaths were from respiratory tuberculosis, and the remaining 8 per cent from other forms of the disease.

Other tissues and organs are involved less frequently. The spleen, liver and kidneys are sometimes infected. Tuberculosis of the adrenals gives rise to Addison's disease. Infection of the skin or lupus is not uncommon. Bone and joint tuberculosis is more common in children than in adults, and tuberculous meningitis is not infrequent in the young. It will be clear that tuberculous infection may take a variety of clinical forms.

The Tubercle. Lesions caused by the tubercle bacillus, in whatever part of the body they occur, usually possess a definite although not absolutely characteristic appearance and histological structure. Small nodules or tubercles, plainly visible to the naked eye, are so uniformly observed in all advanced infections with the tubercle bacillus that their presence has given the name to the disease. The young tubercle probably originates from the fixed cells surrounding the invading bacilli. By the proliferation of the fixed cells, elongated "epithelioid" cells are developed in more or less definite concentric layers and come to form the substance of the tubercle. So called "giant cells" or "foreign

be transmitted by passage to fresh animals (da Rocha-Lima 1920b). The intra-dermal injection of 0.1 ml. of a suspension of infected louse intestines into a rabbit causes a local reaction characterized by redness and infiltration on the 2nd day. The reaction is less severe than that caused by *R. prowazeki*, which often goes on to necrosis, and is neutralized by the serum of a patient convalescent from trench fever (Bieling and Oelrichs 1948). Unlike animals, human beings are almost all susceptible to the disease. One attack seems to produce only a partial and limited immunity; a certain proportion of patients who have recovered from one attack may be reinfected by inoculation of blood or of louse excreta 4 or 5 months later (Bruce 1921).

In 1916 Töpfer (1916a) described the finding of small short rods, often arranged in pairs and often showing bipolar staining, in lice taken from trench fever patients. The rods were shorter and thicker than those seen in typhus, were present in enormous numbers, and were often arranged in heaps. Töpfer fed lice on 7 trench fever patients, and found these bodies in lice taken from 6 of them; they did not appear till the 5th day after feeding, but from the 8th day onwards they were found in large numbers. They were never observed in lice fed on healthy persons. These bodies are now known by the name of *Rickettsia quintana*.

Töpfer's findings have been confirmed and amplified by a number of workers. Arkwright, Bacot and Duncan (1919-20) found that rickettsiae appeared in lice 5 to 12 days after feeding on trench fever patients, and could be demonstrated in the excreta up to the death of the louse. Not all lice became infected at the same time; after feeding on a patient, only a few lice were found to be infected by the end of the 1st week; during the 2nd week the numbers infected and uninfected were about equal, but after the 2nd week the majority showed rickettsiae. The organisms were found in large numbers crowding the region of the epithelial cells lining the gut; they were always extracellular in position. In any given batch of lice rickettsiae developed at about the same time as the louse's excreta became infective—as judged by human inoculation. Moreover a very close correspondence was found between the presence of rickettsiae and the infectivity of the louse, and the absence of rickettsiae and its non-infectivity. Rickettsiae were almost invariably found in batches of lice fed on trench fever patients, but only very rarely when fed on normal persons—one positive result out of 245 specimens.

Rickettsia quintana has never been demonstrated satisfactorily in man, and until this has been done, and until the organism has been grown in tissue culture or the developing chick embryo, it will be impossible to be certain that it is the cause of trench fever. But the observations just quoted on lice strongly suggest that this organism is responsible for the disease.

The remarkable observations made on himself by Bacot (1921) lend support to this view. Bacot went to Poland in February, 1920, with the Typhus Research Commission, taking his own stock of lice with him—a stock that had been under his personal observation and fed upon himself exclusively since November, 1915. During the whole of this time rickettsiae had never been demonstrated in these lice. In April, after visiting a bath-house in Warsaw, he contracted trench fever. Eight days later, he recommenced feeding his lice; rickettsiae indistinguishable from *R. quintana* began to appear, and from the 12th day onwards all the boxes of lice proved to be heavily infected. Bacot's blood remained infective for at least 3 months after the clinical termination of his attack. Of 7 per cent. proved to be infected with rickettsiae, which were indistinguishable from *R. quintana*. Bacot ably become infected from apparently healthy persons who had some time previously suffered from trench fever. The importance of this observa-

body giant cells" soon appear in the developing tubercle which are huge multi-nuclear masses of protoplasm thought by some to be especially distinctive of true tubercle formation, though it is doubtful if this criterion can be maintained. Either they are produced by the fusion of a number of macrophages or they are of single-cell origin. While the formation of epithelioid and giant cells is going on, leucocytes, at first polymorphonuclear leucocytes and later lymphocytes, cluster around the periphery of the tubercle. Degeneration of the tubercle eventually sets in, the central portion becomes necrotic, and this is followed by caseation and then by softening of the caseous mass.

In some cases calcium salts are deposited in the tubercle (calcification), converting it into a hard, dry, friable body which may become encapsulated and completely walled off from the surrounding tissues. In other instances, however, this healing process does not take place, and there is instead an extension with coalescence and the formation of confluent masses that may reach a diameter of 4 or 5 cm. The erosion of a blood vessel may occur, and the discharge of large numbers of the bacilli into the blood stream leads to a general diffusion of small tubercles of the size of millet seeds (acute miliary tuberculosis).

The early stages of tubercle formation, characterized by cell proliferation and leucocytic infiltration, are probably a response to a chemical or mechanical stimulus caused by the presence of the bacilli; the later changes, leading to necrosis and caseation, may be attributed to the action of the bacterial products. The host becomes sensitized to the bacillary substance and the allergic response of the tissues is involved to no small extent.

Predisposing Factors. Few diseases are as completely dominated by predisposing factors as tuberculosis. Infection with the tubercle bacillus is exceedingly common, and there are few adults, particularly those living in cities, who escape. The proportion of individuals coming to autopsy who show evidence of infection is very high and has been reported as high as 97 per cent. Of the more recent reports, for example, Sweany, Levinson and Stadnichenko¹⁴ have found 25 per cent of ten-year-olds, 55 per cent of twenty-year-olds, 80 per cent of thirty-year-olds, and over 90 per cent of individuals over fifty years of age show calcified lesions of infection. The bacilli, then, are present in the great majority of adults and also in many children, but the development of clinical tuberculosis is restrained by the non-specific resistance of the host. Predisposing factors, therefore, are those which tend to interfere with normal physiological well-being and include such things as insufficient or unsuitable food, prolonged exposure to cold, etc. (50)

In the first World War and other countries, and in the second World War the same tendency was again apparent in England.¹⁵ In both man and animals tuberculosis is a disease associated with confinement, of men in houses and cattle kept in stables. The opportunities for transmission of the infection are, of course, much greater in community life.

There is a marked occupational predisposition to tuberculosis in the dusty trades, and the constant inhalation of almost any kind of dust results in an

¹⁴ Sweany, Levinson and Stadnichenko. Amer. Rev. Tuberc., 1943, 48:131.

¹⁵ Med. Res. Council (Great Britain), Spec. Rept. Ser., No. 246, 1942.

TABLE 169
TENTATIVE CLASSIFICATION OF THE RICKETTSIAL DISEASES OF MAN (Modified from Felix 1942a).

Group	Typhus	Spotted fever	Rickettsialpox	Tout-suzamushi	Q fever	Trench fever
NAME OF DISEASE	Classical epidemic typhus Mexican typhus (Tabarillo) Brit's disease Endemic typhus of U.S.A. Australia, Greece, Syria, Manchuria, etc Shup typhus of Malaya Fèvre nautique of Toulon	Rocks Mountain spotted fever (Sato) Brazilian spotted fever (Sato) Paulo typhus Fèvre boutonneuse Kenya fever Tick-borne fever of South Africa, and probably North Queensland tick typhus	Rickettsialpox	Tout-suzamushi scrub typhus of Malaya Mite typhus of Dutch East Indies	Q fever	Trench fever (Wolynian fever, Febria quintana)
VECTOR . . .	—	—	—	—	—	—
ANIMAL RESERVOIR OF VIRUS	—	—	—	—	—	—
VIRUS . . .	—	—	—	—	—	—
	Life and rat fleas					
	Rats	Dogs	Mites	Mites	Ticks	Lice.
	Man	Sheep	Mites and house mice	Voles, Wild rats.	Bandicoots, Opos- sums, Dogs, Cattle, sheep and goats	* Man.
VIRUS . . .	<i>R. prowazeki</i> , <i>R. mooseri</i> Non-filtrable Grows in the cytoplasm No extracellular growth	<i>R. rickettsii</i> , <i>R. rickettsii</i> var <i>conora</i> , <i>R. rickettsii</i> var <i>piperi</i> Non-filtrable Grows mainly in the nucleus No extra- cellular growth.	<i>R. alagzi</i> Non-filtrable. Grows in nucleus as well as in the cyto- plasm	<i>R. nipponica</i> . Non-filtrable Grows only in the cyto- plasm. No extra cellu- lar growth	<i>R. burneti</i> . Filtrable Grows mainly in the cyto- plasm. Some extracellular growth.	<i>R. quintana</i> . Non-filtrable. Not yet cultivated in vitro.
AGGLUTINATION Protein OX	OX 19 + + + OX 2 + OX K —	OX 19 + OX 2 + OX K +	OX 19 — OX 2 — OX K —	OX 19 — OX 2 — OX K + + +	OX 19 — OX 2 — OX K —	*
SUSCEPTIBLE ANIMALS	Febile reaction in guinea- pigs, scrotal reaction with <i>mooseri</i> variety Inapparent infection in rats Classical variety dies out in mice Rabbits insusceptible Cross-immunity between clas- sical and murine types prob- ably not quite complete. Some degree of cross-im- munity between typhus fever and spotted fever	Usually severe disease in guinea-pigs, often with hemorrhagic necrosis of scrotum and ears Febile disease in rabbits. Inapparent disease in mice and rats. Pathogenic to monkeys Cross-immunity complete be- tween Rocky Mountain and Brazilian spotted fever, and flavre boutonneuse; incom- plete with South African tick borne fever	Febile reaction in guinea-pigs with moderate scrotal re- action. Fatal dis- ease in mice. Non- pathogenic to mon- keys	Relatively non-infective for guinea-pigs, but may produce ascites Acute reaction after intra-ocular injection of rabbits Rats and mice difficult to infect Cross immunity com- plete between all three diseases.	Febile disease in guinea-pigs but no scrotal reaction. Inapparent infec- tion in mice. Rabbits insuscep- tible. Cross-immunity be- tween the Australi- an and American diseases complete	Not transmissible to laboratory animals.

increased incidence of pulmonary tuberculosis. Silica dusts, however, appear almost specifically to predispose to tuberculosis and the incidence of infection in those constantly exposed to such dusts is much higher than that in the general population.

It has long been suspected that there are familial or hereditary tendencies to tuberculosis in man. Resistance to infection in experimental animals is to some degree genetically determined, as pointed out elsewhere (p. 217), but the conclusive demonstration of a similar phenomenon in man is difficult owing to both a long generation time and the inability to carry out appropriate breeding experiments. The incidence of new infection in tuberculous families is, of course, considerably greater than in the general population, but whether this is a consequence of increased risk alone or in part of genetically determined factors is not clear. In any case the disease itself is not inherited and congenital tuberculosis rarely if ever occurs.

The relation of childhood infection to clinical tuberculosis in the adult has been a matter of considerable interest, and it has been suggested that pulmonary tuberculosis of the young adult may, in many instances, be a consequence of the lighting up of an old infection. The evidence, however, is against this view, and it is probable that tuberculosis in the adult is a consequence of reinfection rather than of a flaring up of the healed or partially healed lesions from childhood infection. It is to be noted, however, that a considerable period of time, possibly years in some instances, may elapse between reinfection and the appearance of tuberculosis in clinical form.

Bacteriological Diagnosis of Tuberculosis.¹⁶ Tubercle bacilli are discharged from the infected individual in sputum and urine and may be demonstrated in these materials, and in gastric washings, spinal fluid or infected tissues, depending on the location of the infection. Of specimens from these sources, those obtained by gastric lavage are of particular value in respiratory tuberculosis in children, who tend to swallow sputum, and are also useful in adult infections. Results must be interpreted with caution because of the presence of saprophytic acid fast bacilli in the gastro-intestinal tract derived from the ingestion of foods, especially fruit, on which these forms occur, further more, there is some reason to believe that some fats and oils may impart acid fast properties to ordinarily non-acid fast bacteria present in the alimentary tract. The presence of the bacilli may be shown either directly or after concentration by examination of stained smears, culture and guinea pig inoculation.

As a rule, some method of concentration is desirable, for not less than 100,000 bacilli per ml. must be present before there is a reasonable chance of finding them by microscopic examination. Because of the unusual resistance of the tubercle bacillus, the infected material can be treated with an equal volume of 3 per cent sodium hydroxide, or with antiformin (a proprietary preparation of hypochlorite) in final concentration of 15 per cent for fifteen to thirty minutes without killing them. The digested material is neutralized in the case of alkali treatment, centrifuged, and the sediment used for the preparation of smears and to inoculate guinea pigs and culture media. Chemical flocculation

¹⁶For the minimum laboratory standards set up by the American Trudeau Society, given in technical detail, see *Amer. Rev. Tuberc.*, 1942, 45:103.

In practice it is wise to work with suspensions prepared from a non-motile *Proteus* X 19 strain so as to avoid confusion caused by flagellar agglutinins. For this purpose the alcoholized suspensions preserved with formalin, described by Bridges (1935, 1944) and issued in this country by the Standards Laboratory for Serological Reagents at Colindale, are most suitable. Agglutination of a non-motile strain, or the small-flake type of agglutination of a motile strain, is best referred to as agglutination of *Proteus* OX 19. The test itself is usually carried out by the tube method, but for rapid diagnosis and in epidemiological surveys the slide agglutination method may be used (see Eyer and Grutzner 1940, Steuer 1942). The inclusion of a control test made with a standard agglutinating serum is to be recommended (Felix 1950).

So far we have been considering the diagnosis only of the typhus group of fevers by the Weil-Felix test. A modified form of the test is, however, of value in the diagnosis of tsutsugamushi and of the spotted fever group.

Fletcher and Lesslar (1925, 1926), studying the endemic typhus of the Federated Malay States, observed that the Weil-Felix reaction to *Proteus* OX 19 was positive in some cases but not in others. Wondering whether their strain of *Proteus* X 19 was losing its agglutinability, they tried a fresh strain that had been brought out to the Straits Settlements by Dr. Kingsbury from the Middlesex Hospital. This strain, which may be referred to as *Proteus* X K, was found to be agglutinated by the sera of patients which failed to react with the X 19 type. It was further observed that sera which reacted to OX 19 did not agglutinate OX K. There was clearly some distinct serological difference between the two groups of typhus cases. The Kingsbury strain, it may be noted, was reputed to be a strain of X 19, but undoubtedly differed from this type antigenically. Further work (see also Fletcher 1930, 1932) showed that the typhus patients whose blood agglutinated OX 19 were to be found mainly in urban areas, particularly in shops and store-houses, whereas those whose blood agglutinated OX K came from country districts, especially places where rank grass and scrub had grown up on land which had been cleared of jungle. Fletcher referred to the former type of typhus as urban or *shop typhus*, and to the latter as rural or *scrub typhus*.

We have already described how the urban or shop typhus is carried by rats and is of the ordinary endemic murine type, whereas rural or scrub typhus appears to be identical with the tsutsugamushi disease of Japan. Further experience has confirmed the value of the agglutination of OX K in the diagnosis of the tsutsugamushi group of fevers. *Proteus* OX K tends to be agglutinated more often and to a rather higher titre by normal sera than OX 19 (see Report 1942). Special care has to be exercised in the preparation of the suspension (Bridges 1944). If this is properly controlled and other sources of error are excluded, complete agglutination in a final dilution of the patient's serum of 1/200 or a rising titre can generally be accepted as diagnostic of active infection (Felix 1944).

A strain of *Proteus* X corresponding to the virus of the spotted fever group has not yet been discovered, but a reaction, often not appearing till late in the second week, may occur with one or more of the *Proteus* OX 19, OX 2, or OX K strains. Davis, Parker and Walker (1934) found that 77 per cent. of sera taken between the 10th and 32nd days of illness agglutinated *Proteus* OX 19 to 1/160 or over; when two samples were taken at suitable intervals, 95 per cent. of sera proved positive. According to Davis and Parker (1938) a relatively high OX 19 and a relatively low OX 2 titre is the most frequent combination found. Such a result, or agglutination of OX 19 alone, may be due to typhus fever; on the

with alum or ferric chloride is desirable with specimens such as urine or spinal fluid, and may be applied to digested material to facilitate spinning out of the bacilli. A flotation method of concentration may be used which consists of dilution of the digested material with water and shaking with a hydrocarbon such as xylol or gasoline; the creamy layer separating out on standing is smeared and stained.

Smears are usually stained by the Ziehl-Neelsen method; fluorescent microscopy is of sufficiently recent development that it is not yet widely used though it may well become generally adopted. The demonstration of acid-fast bacilli allows a provisional diagnosis but does not indicate whether the bacilli were viable, or whether they are virulent tubercle bacilli. Their presence in urine specimens in particular must be interpreted with caution because of the frequent occurrence of the smegma bacillus which is also acid-fast.

Guinea pigs are inoculated in the groin or in the muscle of the thigh. If a reasonable number of tubercle bacilli have been inoculated, enlargement of the regional lymphatics may be noted in two or three weeks, the pig becomes emaciated by four to six weeks and usually dies not long after. With very small numbers of bacilli evidence of the infection may be delayed two or three weeks longer. If it is not obviously ill or does not die, the animal should be kept for eight weeks before sacrificing. At autopsy the regional lymph nodes will be found to be enlarged and filled with caseous material. Necrotic areas in the spleen and liver are characteristic of the gross pathology in this animal; tubercles are seldom seen, and the lungs are only slightly affected and the kidneys almost never. Tubercle bacilli may be cultured from the lesions and found in acid-fast-stained smears. Some workers tuberculin-test the animals before inoculation and three to four weeks afterward, the development of a hypersensitivity indicating infection. If differentiation of the human and bovine varieties of the bacillus is desired, rabbits may be inoculated.

The culture of tubercle bacilli, carried out concurrently with animal inoculation, is becoming quite general and some workers feel that it is as sensitive as guinea pig inoculation. As indicated earlier, a variety of media, containing glycerol and egg, may be used, the particular medium varying from one laboratory to another. A slant is inoculated heavily with the sediment from the digested specimen and, after flaming, the plug is pushed into the tube to leave about 5 mm. empty space above it and warm paraffin poured in to a depth of 2 to 3 mm., allowed to harden and a second application made. After this hardens a hole is punched through to the plug, allowing an adequate gas exchange while preventing excessive dehydration of the medium. Characteristic colonies of tubercle bacilli appear after about three weeks' incubation. The liquid medium of Dubos is probably the most sensitive providing that the lipase present in the usual bovine albumin preparations is eliminated. Dubos and Davis¹⁷ have reported that inoculums as small as two cells grow regularly. Preliminary tests with tuberculous specimens have given suggestive results.¹⁸ Culture is not identification, of course, and acid-fast bacilli are occasionally found in non-tuberculous lesions which grow like the tubercle bacilli.¹⁹

¹⁷ Dubos and Davis: *Jour. Bact.*, 1948, 55:11.

¹⁸ Foley: *Proc. Soc. Exp. Biol. Med.*, 1946, 62:298; Goldie *ibid.*, 1947, 65:210.

¹⁹ See the discussion by Corey. *Amer. Rev. Tuberc.*, 1945, 52:36

demonstrated by mixing the patient's serum with a suspension of living rickettsiae, incubating at 37° C. for 30-60 minutes, and injecting the mixture into the skin of a guinea-pig. With a control serum a reaction is evident 4-7 days later: with a serum containing neutralizing antibodies the reaction is more or less completely suppressed. Antibodies may be demonstrable years after an attack of the disease (Giroud 1938).

Discussion of the Weil-Felix Reaction.

The explanation of this reaction is still obscure.

That the *Proteus X* bacilli are not themselves responsible for the disease is indicated by the following facts: (1) they are found in only a minority of typhus patients; (2) they do not produce experimental typhus in the guinea-pig; (3) they are never isolated from guinea-pigs infected with true typhus virus; (4) guinea-pigs inoculated with *Proteus X* bacilli develop no immunity to the subsequent inoculation of typhus virus; (5) guinea-pigs that have recovered after inoculation of typhus virus are as susceptible as normal guinea-pigs to a lethal dose of *Proteus X* bacilli (Otto 1919).

Felix himself is of the opinion that the two organisms are genetically related and that *Proteus X* must be regarded as a variant of *Rickettsia*, differing from this organism in its morphological, cultural, and pathogenic properties, but resembling it in the possession of a common O antigen (see Chapter 39 and Felix and Rhodes 1931, Felix 1933a, b, 1935). If this is so, then *Proteus X* 19 may be looked upon as the variant of *R. prowazeki*, and *Proteus X* K as the variant of the *Rickettsia* of scrub typhus. *Proteus X* variants corresponding to *R. rickettsi*, *R. burneti*, and *R. quintana* have not yet been discovered, though it is interesting to note that strains with a dual antigenic structure responding to both OX 19 and OX K agglutinins have been reported in Tunis (type S 24) and in São Paulo (type X L) (see Laigret and Durand 1934).

We should be unwise, however, to accept such an explanation without definite proof. Clearly, the most satisfactory evidence would be afforded by a direct demonstration of the change from *Rickettsia* to *Proteus*, either in tissue culture or in the louse's intestine. Attempts to prove the existence of a genetic relationship along these lines have not yet proved successful, though suggestive results have been recorded by Weigl (1923), Anigstein (1933), and Anigstein and Lawkowiez (1933).

As a corollary it would be important to show that *Proteus X* strains were never encountered except in relation to *Rickettsia*. Our available information on this point is not very reassuring.

A number of workers (see Sparrow and Roussel 1936, 1937, van Loghem 1936) have isolated *Proteus X* 19 strains from persons suffering from diseases other than typhus. Sparrow and Roussel, working in Tunis where typhus is epidemic, cultivated organisms of the X 19 type from the blood of about 50 per cent. of typhus patients in 1935, and from about 9 per cent. of patients suffering from other diseases, such as undulant fever, typhoid fever, malaria, and pulmonary tuberculosis; in the following year, however, the proportion of positive cultures was very much lower. It may further be noted that *Proteus* strains have been found in the blood and urine of typhus fever patients which were not of the X 19 type (Schürer and Wolff 1919; see also Felix 1931).

Some workers, such as Otto (1919), have regarded the Weil-Felix reaction as an example of para-agglutination, using this term to mean the agglutination of a heterologous organism by virtue of a property conferred on it by contact with the homologous organism. It is supposed that *Proteus* bacilli in the body of a typhus-infected patient gain new antigenic receptors, which render them susceptible to antibodies produced against the typhus virus. If this were true, it

Immunity. The immune response of the animal body to the presence of the tubercle bacillus is indicated by the appearance of agglutinins, precipitins, opsonins and complement fixing antibodies in the serum. This response is not marked, however, for these antibodies are present only to low titers. None of them, with the possible exception of the complement-fixing antibodies, is of diagnostic value.²⁰

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ments. He showed that the subcutaneous inoculation of the normal guinea pig with tubercle bacilli produces no immediate response but that in ten to fourteen days a nodule develops which breaks down to a persistent tuberculous ulcer and the regional lymph glands become swollen and caseous. In the tuberculous animal, however, an indurated area appears within a day or two, and there is slight necrosis with the formation of a shallow ulcer which heals promptly without the development of gross tubercle tissue or the invasion of the adjacent lymphatics by the bacilli. This resistance to reinfection is relative, of course, and is not shown unless the primary infection is of some weeks' standing or if large numbers of bacilli are injected. Animals may be sensitized, not only by infection with virulent bacilli, but also by the inoculation of attenuated or killed bacilli. Sensitization with preparations of the bacillary cell substance is difficult, however, and very large doses must be administered. Raffel²¹ has shown that the delayed, non-passively transferable reaction is a result of inoculation with tubercle bacillus protein combined with a purified wax fraction consisting of an ester of polysaccharide and higher alcohols with hydroxy fatty acids, the protein alone produces an immune response with formation of precipitins.

Tuberculin.²² The sensitized animal will react to the soluble cell substance of the tubercle bacillus, preparations of which have been called *tuberculin*. Tuberculin is usually prepared from the human type of tubercle bacillus, though bovine tuberculin is practically as active as human tuberculin in infections with the human bacillus; avian tuberculin, although considerably less active, will also produce a reaction. A variety of tuberculins have been prepared of which only a few need be noted here. The first of these was made by Koch, and consisted of the filtrate of a glycerol broth culture of the bacilli concentrated by evaporation on a water bath to about one tenth its original volume (the activity is heat-stable). This material is "original" or "old" tuberculin (TO or OT). A "new" tuberculin (TR—tuberculin residuum) was prepared by Koch in 1897 by macerating living virulent bacilli and extracting the mass with water, and then making an emulsion of the residuum. He later advocated the use of an emulsion (BE—bacillary emulsion or *Bazillenemulsion*) of the entire substance of young virulent bacilli in 20 per cent glycerol, actually a vaccine. Denys introduced the use of the undiluted filtrate from broth cultures (BF—broth filtrate). None of these later innovations, however, proved to be superior to old tuberculin, and the original preparation, or slight modifications of it, has been widely used.

²⁰ Cf. Klepzig, *Brit. Jour. Tuberc.*, 1911, 35:146.

²¹ *Raffel Jour. Inf. Dis.*, 1918, 82:267.

²² Cf. the discussion by Parish, *Tubercle*, 1938, 19:337.

agents, and partly because of the sporadic nature of the disease in man. In murine typhus fever the usual measures may be taken for the suppression of rats, and DDT may be applied to rat runs and holes to destroy the fleas. Against tsutsugamushi the most valuable general measure has proved to be impregnation of the clothing with dibutyl phthalate or benzyl benzoate to protect against mites (McCulloch 1946, Bushland 1946). No satisfactory insecticide to repel ticks is yet known, but in suitable circumstances attempts may be made to free infested animals by dipping them in a solution of sodium arsenite or spraying them with DDT or gammexane. Ticks attach themselves to man rather slowly and do not transfer infection at once, so that in tick-infested areas an examination should be made of the body and clothing twice daily to remove any ticks that may have gained access to them. Precautions may have to be taken to prevent the inhalation of infected dust arising from louse faeces or other material. To prevent Q fever spread by infected milk, pasteurization is desirable, preferably at a temperature of 162° F. for 15 seconds (see Huebner *et al.* 1949, Marmion *et al.* 1951).

Vaccination.

Numerous attempts have been made to produce active immunity against typhus fever by vaccination. Some workers have used live vaccines. Nicolle and Laigret (1936), for example, prepared a vaccine of murine virus from infected guinea-pig and rat brain, and Clavero del Campo and Gallardo (1949) a vaccine of an avirulent variant of the epidemic virus. These products are not without danger, and most workers prefer killed vaccines. For these a thick suspension of rickettsiae is required, as Fulton and his colleagues (1946) have shown that, no matter how the vaccine is made, its protective potency depends on its rickettsial content. Various methods have been used.

(a) *Weigl's louse vaccine*.—Lice are inoculated by the rectum with the classical typhus virus. After a week or so they are killed. The intestines are removed and ground up with 0.5 per cent. phenolized saline (Weigl 1933). Since three doses are given, requiring the use of 120–175 lice for each subject, an enormous louse farm has to be maintained if vaccine is to be prepared on any considerable scale. An additional drawback is the necessity for feeding lice on immune persons for a week after infection.

(b) *Killed suspensions of rickettsiae grown in animals*.—The early method of Zinsser and Castaneda (1930a, 1931, 1933) of using a murine virus injected intraperitoneally into guinea-pigs or rats whose resistance had been lowered by irradiation or other means was abandoned when Okamoto (1937) and Castaneda (1939) showed that abundant growth of rickettsiae occurred in the lungs of mice and rats injected intranasally with the murine virus. A vaccine can be prepared by grinding up the lungs 3 days after inoculation, killing the rickettsiae with phenol or formalin, and concentrating the organisms by differential centrifugation (Castaneda 1939, Durand and Giroud 1940a). Three doses of vaccine are recommended for man at 7-day intervals (Castaneda 1941). The mouse's lung can also be used for growing an adapted strain of the classical typhus virus, and Castaneda (1948) described a method for preparing by this means a bivalent vaccine containing *R. prowazeki* and *R. mooseri*. He recommended the injection of 5 or 6 doses at weekly intervals.

(c) *Killed suspensions of rickettsiae grown in tissue culture*.—Zinsser, Fitzpatrick and Wei (1939) described a method of growing rickettsiae in a modified Maitland medium making use of agar blocks covered with living tissue. The growth can be removed and treated with 0.3 per cent. formal for conversion into a vaccine.

(d) *Chick embryo vaccine*.—Cox (1938, 1941) found that much the best method of growing rickettsiae of typhus fever, spotted fever and Q fever was in the yolk sac of the developing

The active principle of tuberculin is protein in nature, and the cultivation of the tubercle bacillus in glycerol-asparagin-citrate synthetic solutions by Long and Seibert has made possible the study of the active principle in purified preparations. The activity is associated with a number of protein fractions, one of which Seibert prepared in crystalline form. A more satisfactory preparation of low molecular weight, ca. 2000, has been isolated by Seibert by precipitation with trichloroacetic acid. Originally designated SOTT (synthetic medium old tuberculin trichloroacetic acid precipitated), it is now known as PPD (purified protein derivative).²³

The comparative merits of OT and PPD have been the subject of a series of investigations in recent years. OT is relatively unstable in dilutions, while PPD, a dry powder, is "dry-diluted" with lactose and is, of course, indefinitely stable in this form. Different lots of OT vary somewhat in their activity; the activity of PPD preparations is relatively constant. It appears that PPD is quite as satisfactory as OT in actual use and, because of its stability and constant activity, is regarded by many as superior to OT.

*The Tuberculin Reaction.*²⁴ Three types of reaction may be elicited in the sensitized, i.e., infected, animal by the injection of tuberculin. In addition to a local inflammatory reaction at the site of inoculation, there is a focal reaction manifested as an acute congestion around tuberculous foci which, if marked, may aggravate the pathologic process, and a constitutional reaction in which the temperature rises to a peak of 102° to 104° F. and subsides in twelve to eighteen hours. In man the constitutional reaction also includes malaise, pain in the limbs and, perhaps, vomiting, dyspnea and other symptoms. These reactions do not appear in normal animals. The utility of tuberculin is, then, twofold; it may be used for diagnostic purposes and it has therapeutic value, though the latter is strictly limited.

The diagnostic tuberculin test in man is generally a skin test. Koch's original method consisted of subcutaneous injection of tuberculin. The cutaneous reaction of von Pirquet involves the rubbing of tuberculin on to the scarified skin. In the Mantoux test, the one most commonly used today, graded doses of tuberculin are injected intradermally, usually starting with 0.01 mg. of OT and going as high as 1.0 mg. or even 10 mg. on rare occasions. (0.1 ml. of a 1:100 dilution of OT is supposed to contain 1 mg.; the standardization of new batches is biological and carried out in guinea pigs infected with virulent tubercle bacilli.) Smaller amounts of PPD are used, since it is in dry, pure form; usually 0.00005 to 0.005 mg. A "patch test" has been introduced by Vollmer²⁵ in which squares (0.8 cm.) of thin filter paper, impregnated with tuberculin about four times as strong as the original old tuberculin and dried, are taped on the cleansed skin over the sternum or upper edge of the trapezius. The patch test appears to be somewhat less sensitive than the intracutaneous test of Mantoux. Since these are all skin tests only the local inflammatory reaction is observed in infected persons.

²³ See the reviews by Seibert: *Amer. Rev. Tuberc.*, 1941, 44:1; *Bact. Rev.*, 1941, 5:69. *Chem. Rev.*, 1944, 34:107.

²⁴ See the general comprehensive discussion by Long: *Amer. Rev. Tuberc.*, 1939, 40:607.

²⁵ Cf. Vollmer and Goldberger: *Amer. Jour. Dis. Children*, 1937, 54:1019, *ibid.*, 1938, 56:584, *ibid.*, 1939, 57:1272, *ibid.*, 1939, 58:527, *Mandel Arch. Pediatrics*, 1945, 62:393.

Chemoprophylaxis and Chemotherapy.

Little is known about the prophylactic effect of drugs on the typhus group of fevers except for tsutsugamushi. In the prevention of this disease Smadel and his colleagues (1949) showed in a controlled investigation that 1 gm. of chloramphenicol given daily protected exposed human volunteers during the time the drug was taken and for about a fortnight afterwards.

In the treatment of the typhus fevers various dyes such as methylene blue and toluidine blue, members of the acridine series, and *p*-aminobenzoic acid have all been shown by egg culture or animal experiment to possess an anti-rickettsial action (for references see Findlay 1948), but their value in practice is overshadowed by the antibiotics chloramphenicol and aureomycin. The remarkable therapeutic effect of chloramphenicol on tsutsugamushi was demonstrated by Smadel and his colleagues (1948) in Malaya, where it was found that the temperature usually fell to normal within 24-48 hours of the beginning of treatment. The effect of the drug is to suppress rather than to kill the rickettsiae, so that relapses occur in a high proportion of patients a week or so after it is discontinued; they can, however, be prevented or cured by reinstitution of treatment (Smadel, Bailey and Diercks 1950). Chloramphenicol also appears to be of value in the treatment of epidemic typhus fever and of Rocky Mountain spotted fever. Aureomycin is likewise reported on favourably in the treatment of this latter disease. For Q fever terramycin appears to be the drug of choice.

Serum Treatment.

Nicolle, Conor and Conseil (1911) found that the serum of human patients or monkeys convalescent from typhus fever possessed protective and curative properties. Durand and Balozet (1940, 1941) prepared antiserum by the inoculation of horses and asses with mouse lung suspensions of the murine and classical types, and by animal experiments showed it to be more potent than human convalescent serum. A similar antiserum was prepared by Topping (1943) for the treatment of Rocky Mountain spotted fever by the inoculation of rabbits with infected ticks or yolk-sac cultures. None of these antisera has ever been tested under properly controlled conditions, and it is doubtful whether, with the advent of chloramphenicol, they are likely to be used in practice again.

RICKETTSIAL INFECTION OF ANIMALS

Though animals often act as a reservoir of rickettsiae that are pathogenic for man, little is known as yet of the occurrence of animal disease due to other types. The best example is heartwater.

Heartwater.—Economically, this is an important disease of sheep, goats, and cattle. It is widespread over the whole of Africa, except the northern portion. Infection is due to *Rickettsia ruminantium*, first described by Cowdry (1925), and is transmitted by the tick *Amblyomma hebraeum*. Clinically, the disease varies from the hyperacute form, on the one hand, to the abortive form, on the other. The incubation period in sheep is 7 to 14 days. The acute form is characterized by fever, serous effusions in the pleura, pericardium, and peritoneum, and convulsive symptoms. The course is 2-6 days, and the case fatality 50-100 per cent. Cases occur sporadically and in small groups, not in large epidemics (Donatien and Lestoquard 1937). *R. ruminantium*, like the organisms of typhus and of spotted fever, infects primarily the vascular endothelium, and like *R. prowazeki*,

In young children a positive tuberculin reaction may be taken as indicative of infection. It was formerly believed that, once established, the hypersensitivity persists essentially throughout life and that the tuberculin reaction is of limited value in the adult. It is becoming apparent, however, that reversion is more frequent than had been generally realized, particularly with reduction in the prevalence of the disease and therefore the risk of reinfection. Furthermore, there is some evidence that the correspondence of a positive tuberculin reaction and either healed or clinically significant tuberculous infection may not be as close as generally believed.²⁶

The tuberculin test in cattle has great diagnostic importance, however, and has been widely used in the United States and to a lesser extent elsewhere. Three types of test may be used in cattle—the intradermal test, the ophthalmic reaction of Calmette, in which tuberculin is dropped into the conjunctiva and the reactive animal responds with the development of a diffuse congestion and edema in six to eight hours which fades away in twenty-four to thirty-six hours; and the constitutional reaction as indicated by a rise in temperature following the injection of tuberculin. The intradermal inoculation into the skin of the caudal fold is generally practiced in this country.

The therapeutic value of tuberculin may be directly observed in lupus or tuberculous infection of the skin. As indicated above, there is a reaction about the foci of infection manifested as an acute congestion and sloughing off of tissue. When it was first introduced tuberculin was regarded by many as a highly effective specific therapeutic agent for tuberculosis. Its use is, however, exceedingly dangerous, and, with the exception of lupus, the response of tuberculous infection to the injection of tuberculin has been disappointing. The therapeutic use of tuberculin has diminished greatly in recent years. It may be noted that the minute quantities used in the tuberculin test do not affect tuberculous lesions.

The Mechanism of Immunity. Infection with the tubercle bacillus confers a definite protection against reinfection, as "immunity to superinfection" resembling that observed in syphilis. The factors involved are as yet obscure. Some current opinion favors the view that the development of an allergic state is indicative of an effective immunity.²⁷ In this connection Brosius and Woodruff²⁸ have pointed out what has been casually noticed by many workers, namely that in individuals giving a positive tuberculin reaction living cells are usually free of tubercle bacilli and the bacteria are present in necrotic areas separated by an avascular barrier, while in the infected individual giving a negative reaction, tubercle bacilli are found in great numbers in living tissue. The antibodies produced are apparently not significant and antisera have no protective or curative properties. As indicated above, the development of hypersensitivity is the most obvious response to infection, and there is no doubt that hypersensitivity plays a part in acquired resistance as indicated in Koch's early experiments. Its relative importance is, however, not at all clear, and the

²⁶ Lumsden, Deating and Brown. *Amer. Jour. Pub. Health*, 1932, 22:25; Dahlstrom. *Amer. Rev. Tuberc.*, 1940, 42:471.

²⁷ Lurie. *Bull. Off. Internat. d'Hyg. Publ. Que.*, 1946, 35:1021; Negre and Boretex. *ibid.*, 1946, 35:1034.

²⁸ Brosius and Woodruff. *Amer. Rev. Tuberc.*, 1944, 50:473.

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mechanism of what seems to be a low-grade immunity of short duration remains at the moment largely a matter of speculation.

Active Immunization. The possibility of active immunization to tuberculosis has been of great interest since the discovery of the tubercle bacillus. In general, two types of vaccines have been employed, suspensions of living attenuated bacilli and suspensions of killed bacilli.

The attenuated bovine strain of Calmette, BCG, has been regarded as the most promising immunizing agent and has been extensively studied. It was originally given as an oral vaccine in France in the early 1920's. However, the combination of inadequate statistical data and an incident in Lubeck, Germany, in which a virulent strain was inadvertently substituted for the vaccine strain, resulting in tuberculosis in inoculated persons, put it into disrepute. Nevertheless immunization with BCG was further studied in the Scandinavian countries, beginning in 1925 in Sweden and in 1927 in Norway and Denmark. The vaccine is given intracutaneously in doses of 0.05 to 0.15 mg. and a positive tuberculin reaction appears in six to ten weeks in over 90 per cent of those inoculated. The hypersensitivity lasts for about four years, though in some persons there is reversion in as little as one year. On the assumption that a positive tuberculin reaction is indicative of immunity, reversion is taken to mean that reinoculation is required. Sufficient time has now elapsed to permit judgment as to the harmlessness and efficacy of BCG vaccine. Swedish experience is summarized by Wallgren,²⁹ Norwegian by Hansen,³⁰ and Danish by Holm,³¹ and that of the Scandinavian countries as a whole by Birkhaug.³² In addition to these, shorter term immunization studies have been carried out with nurses in Canada by Ferguson,³³ and in the United States with American Indians by Aronson and Palmer³⁴ and with infants by Rosenthal.³⁵ In general the foregoing studies have indicated that the immunization confers an appreciable, but not absolute, degree of protection against primary infection which is somewhat less than that given by the natural infection. For example, in the group studied by Aronson and Palmer the total incidence per 1000 person-years in the six year period of observation was 24.3 and 4.7 in the control and immunized groups respectively. Perhaps more important is the apparently high degree of protection against the immediate consequences of primary infection and the more severe forms of the disease.

These conclusions are not universally accepted without reservation. These recent reports have been considered critically by Wilson,³⁶ who is not so optimistic and emphasizes the living nature of a vaccine whose virulence is not fixed, the necessity of separation of the inoculated infant for some weeks before and after vaccination, the immaturity of the immunity mechanism at birth, etc., as deterrents to a general application of the immunization procedure in the more highly civilized countries. In the United States the reported results

²⁹ Wallgren: Bull. Office Internat. d'Hyg. Publique, 1946, 38:1052.

³⁰ Hansen: Tubercle, 1944, 25:1.

³¹ Holm: Pub. Health Repts., 1946, 61:1298.

³² Birkhaug: Amer. Rev. Tuberc., 1947, 55:234.

³³ Ferguson: Amer. Rev. Tuberc., 1946, 54:325.

³⁴ Aronson and Palmer: Pub. Health Repts., 1946, 61:802.

³⁵ Rosenthal, Leslie and Loewinsohn: Jour. Amer. Med. Assn., 1948, 136:73.

³⁶ Wilson: Brit. Med. Jour., 1947, 11:855.

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are regarded as highly suggestive of the efficacy of such immunization, but BCG vaccination is considered to be in only an experimental stage as yet.³⁷

Perhaps to be regarded in the category of attenuated living bacilli is the "turtle bacillus" vaccine of Friedmann.³⁸ This microorganism, *Mycobacterium chelonae*, is of the "cold blooded" variety and was isolated from a turtle. It is quite ineffective as an immunizing agent and is of only historical interest.

Suspensions of killed tubercle bacilli have been used in the immunization of cattle and experimental animals. The Spahlinger vaccine³⁹ is of this type. Although the resistance of guinea pigs and cattle appears to be raised by inoculation, the "immunity" so produced is of a very low order.⁴⁰ The inoculation of human beings with suspensions of killed bacilli has not been attempted on a large scale.

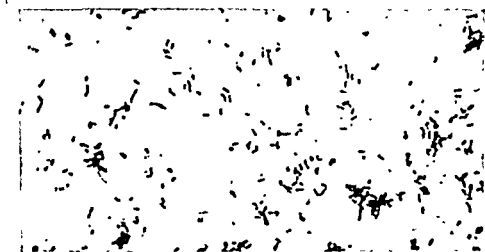


Fig. 148. Avian tubercle bacillus. Acid fast stain of a smear from a pure culture. $\times 1050$.

Pathogenicity for Lower Animals. Tuberculosis of lower animals living under natural conditions is probably very rare. Animals in captivity, however, may contract the infection with some facility; in animals kept in zoos and in monkeys in the laboratory for experimental purposes tuberculosis is not uncommon. Domestic animals may be similarly affected. A wide variety of animals may, of course, be infected experimentally with one or another of the types of tubercle bacilli.

Domestic Animals. The most commonly infected domestic animals are cattle, pigs and chickens. Cattle are infected with the bovine type of tubercle bacillus almost exclusively, they are not completely resistant to the human type, as Koch originally thought, but infection is accomplished with some difficulty. The proportion of cattle infected increases with advancing age and, in the absence of control measures, may reach 70 to 90 and possibly 100 per cent in animals

³⁷ Cf. Pub. Health Repts., 1947, 62 316.

³⁸ Cf. Friedmann and Aronson, Jour. Inf. Dis., 1927, 44 222.

³⁹ Spahlinger, Lancet, 1932, i 369.

⁴⁰ Cf. Report on the Spahlinger Experiments in Northern Ireland, H. M. Stationery Office, London, 1935.

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kept in stalls. The natural infection is generally of a chronic, slowly progressive nature. The lymphatics are most frequently involved and may be the only tissues to show lesions. The lungs are also commonly affected. Lesions on the pleura have a peculiar characteristic appearance, the so-called "perlsucht" disease. The liver, spleen and kidneys are less frequently involved, but infection of the mammary glands is not uncommon. It may be noted that tubercle bacilli may be excreted in the milk in the absence of detectable lesions of the mammae. Congenital tuberculosis in cattle occurs with some frequency.

Tuberculosis in chickens is very common and is exclusively an infection with the avian variety of the tubercle bacillus. With the exception of parrots and certain birds of prey, birds are highly resistant to infection with the human and bovine varieties, and it is probable that natural infection with these types rarely if ever occurs. Tuberculosis in chickens is usually a chronic process and is characterized by the formation of nodules in the abdominal viscera. The lungs are less frequently affected.

CHARACTERISTICS OF THE VARIETIES OF TUBERCLE BACILLI

Variety	Physiology				Pathogenicity		
	Opt temp	Rate of growth	Pigment	Stimulation by glycerol	Guinea pig	Rabbit	Chicken
Human	37° C	eugonic	+	+	++++	±	-
Bovine	37° C	dysgonic	-	-	++++	++++	-
Avian	40-42° C	rapid	+	+	+	++	++++

Pigs suffer from natural infection with both bovine and avian tubercle bacilli derived from infected cattle and poultry; they are also susceptible to infection with the human variety. In young pigs infection with bovine bacilli is generalized and acute with lesions in the lymphoid tissue, abdominal viscera and lungs. Infection with the human bacillus is generally localized, but the avian type may produce a generalized infection.

Other domestic animals suffer from tuberculosis to a considerably lesser extent. Horses, dogs and cats are occasionally infected, and the disease is rare in sheep and goats.

Experimental Animals. Experimental animals vary in their susceptibility to the varieties of the tubercle bacillus and in the type of infection produced. Of these animals only two need be considered here, the guinea pig and the rabbit. The guinea pig is highly susceptible to both bovine and human bacilli, and death follows the subcutaneous injection even of small doses in six to fifteen weeks. The lymphatic glands, spleen and liver are most affected, the lungs only slightly and the kidneys never. The necrotic areas in the spleen and liver are the most striking feature of the gross pathology and are peculiar

it is not always successful, owing to the long incubation period of the disease and other causes. (For pleuropneumonia of calves due to *Pasteurella septica*, see p. 1855)

CONTAGIOUS AGALACTIA OF SHEEP AND GOATS

This disease is characterized by inflammatory lesions of the udder, eye, and joints. The milk yield in lactating animals is diminished, and instead of milk, a dirty yellow serous fluid containing small clots is secreted (Galloway 1930). Several outbreaks have occurred during the past hundred years in Europe, and more recently the disease has been encountered in North Africa. The case fatality is said to average about 15 per cent. The causative organism, which was first cultivated by Bridré and Donatien (1923, 1925), resembles the organism of pleuropneumonia in its pleomorphism and its filtrability. Natural infection usually appears to occur by ingestion. The disease can be reproduced by inoculation, especially of goats, with infected tissue suspensions, and with pure cultures (see Chapter 40). Animals that have recovered naturally from an attack of the disease have a high degree of immunity. Active immunization is very difficult. Passive immunity may be conferred by the injection of immune sera prepared in sheep, goats, or horses (Bridré and Donatien 1925).

DISEASES CAUSED BY OTHER MEMBERS OF THE PLEUROPNEUMONIA GROUP OF ORGANISMS

Numerous diseases of animals have now been ascribed to infection with other members of the pleuropneumonia group, such as arthritis, lymphadenitis, and suppurative lesions in rats, and arthritis and rolling disease in mice. The relation of these organisms to bronchiectasis in rats, distemper in dogs, and certain genital lesions in human beings is still under discussion. Space does not allow a full description of each of these diseases; adequate attention has already been paid to them in Chapter 10, which the reader may consult for references to the original papers.

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to the guinea pig. True tubercles are seldom seen except in the very early stages of the disease.

Rabbits are highly susceptible to infection with the bovine bacillus, somewhat so to the avian bacillus and quite resistant to the human variety. Injection of bovine bacilli produces a generalized infection that terminates fatally in two to three months. On autopsy tubercles may be found in the spleen and liver, but the lesions are most marked in the lungs and kidneys and may even be confined to them. Very large doses of human bacilli (10 to 50 mg. intraperitoneally) may produce a progressive infection but never the acute

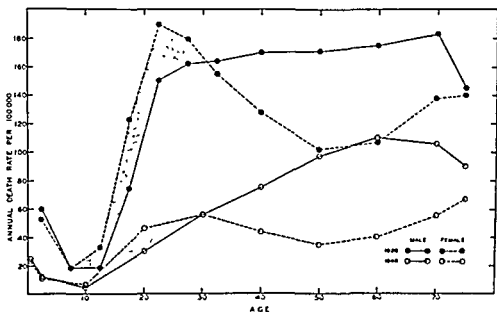


Fig. 149. The age and sex distribution of tuberculosis mortality (all forms) in 1920 and 1940. Note the differential incidence and decline over the twenty year period. If differentiation between white and colored rates is made for 1940, the picture is almost the same, i.e., the colored rates are very similar to the 1920 rates for both white and colored. Data from the reports of the Census Bureau.

fatal military disease. The mammalian tubercle bacilli, indistinguishable by cultural or serological methods, may, then, be sharply differentiated by their pathogenicity for these two experimental animals.

Neither rabbit or guinea pig is particularly susceptible to infection with the avian bacillus though avian tuberculosis may be produced in the rabbit.⁴¹ In the guinea pig death may be produced by large intraperitoneal doses, but on autopsy microscopic tubercles are not visible, cultures and smears of the liver and spleen, however, show the presence of the bacilli. This form of tuberculosis, the proliferation of the bacilli without macroscopic tubercle formation, is known as the *Yersin* type of tuberculosis.

Epidemiology. In man tuberculosis is largely an airborne infection. Dissemination by milk is of relatively minor importance, particularly in the United States. The disease is one of civilization in that its transmission is

⁴¹ See Feldman: *Avian Tuberculosis Infection*. Williams and Wilkins Co., Baltimore, 1943.

is contracted through sexual intercourse. After healing of the initial penile lesion, males cease to be infectious, whereas females, on account of the peculiar lymphatic distribution in the perineal region, remain infectious for months and years. In females particularly, the disease often affects the genito-ano-rectal tissues with the production of a stricture—a condition referred to as *esthiomène*—though it is not yet established that all cases of *esthiomène* are caused by the lympho-granuloma virus.

Attention was first concentrated on the local lesions, but the disease is now known to be accompanied by a generalized infection. In its early stages it may even simulate typhoid fever; arthritis and skin rashes are not uncommon, and symptoms suggestive of meningeal affection may occur (Findlay 1938). Congenital transmission of maternal antibodies has been noted, though the virus itself does not appear to pass through the placenta (Levine *et al.* 1943). A few laboratory infections have been reported (Harrop *et al.* 1941). In adults infection may remain latent for months or years. Pathologically, the essential lesion produced by the virus is a low-grade inflammation of the lymph spaces and lymph channels leading to caseation and fibrosis.

Intracerebral inoculation of infective material into monkeys gives rise, after an incubation period of 6–12 days, to meningo-encephalitis. The mouse is also susceptible; after intracerebral inoculation, symptoms appear in 2–4 days; the coat is roughened, the back is hunched, and purulent conjunctivitis may be seen; death occurs in 3–8 days. The virus may be carried on indefinitely by passage, but some strains gradually become inactive. According to Miyagawa and his colleagues (1936a), their virulence may be restored by intradermal passage through guinea-pigs, in which the virus produces a caseous papule at the site of inoculation. Intranasal inoculation of mice may give rise to focal lesions in the lungs (van den Ende and Lush 1943). Guinea-pigs and rabbits are relatively resistant to intracerebral infection. Infected monkey or mouse brain, when inoculated on to the prepuce, is able to reproduce the typical disease in human subjects (Levaditi *et al.* 1932, 1935, Wassén 1935).

Ætiology.—The causative virus passes through Berkefeld N and Chamberland L3 candles, though the results are said to be variable. According to Miyagawa and his colleagues (1935c) and to Broom and Findlay (1936), its probable diameter is about 0.125–0.175 μ , but electron microscope studies place it nearer 0.45 μ (Kurotchkin *et al.* 1947). It is destroyed by exposure to a temperature of 56° C. for 10 minutes, and is readily inactivated by glycerol and by 0.1 per cent. formol. At 4° C. it remains virulent for about 3 weeks. The virus is difficult to grow in tissue culture (Miyagawa *et al.* 1936b), but it can be propagated indefinitely by passage through the yolk sac of the developing chick embryo (Rake *et al.* 1940, 1941). An infection occurs that proves fatal to the embryo in 3 days to 2 weeks. Virus is present in large quantity in the wall of the yolk sac and in the yolk itself. Elementary bodies, demonstrable by histological methods, have been described by various observers in infected tissue from man, monkeys, and mice (for references see Findlay *et al.* 1938). They are sometimes referred to as the granulo-corpules of Miyagawa (Miyagawa *et al.* 1935a, b). Neutralizing antibodies may be detected in the serum of patients suffering from the disease and in the serum of inoculated rabbits. According to Rake and Jones (1944) the virus is able to give rise, when grown in the yolk sac of the chick embryo, to a thermolabile endotoxin killing mice, injected intravenously, in 12 to 24 hours.

kept in stalls. The natural infection is generally of a chronic, slowly progressive nature. The lymphatics are most frequently involved and may be the only tissues to show lesions. The lungs are also commonly affected. Lesions on the pleura have a peculiar characteristic appearance, the so-called "perlsucht" disease. The liver, spleen and kidneys are less frequently involved, but infection of the mammary glands is not uncommon. It may be noted that tubercle bacilli may be excreted in the milk in the absence of detectable lesions of the mammae. Congenital tuberculosis in cattle occurs with some frequency.

Tuberculosis in chickens is very common and is exclusively an infection with the avian variety of the tubercle bacillus. With the exception of parrots and certain birds of prey, birds are highly resistant to infection with the human and bovine varieties, and it is probable that natural infection with these types rarely if ever occurs. Tuberculosis in chickens is usually a chronic process and is characterized by the formation of nodules in the abdominal viscera. The lungs are less frequently affected.

CHARACTERISTICS OF THE VARIETIES OF TUBERCLE BACILLI

Variety	Physiology				Pathogenicity		
	Opt temp	Rate of growth	Pigment	Stimulation by glycerol	Guinea pig	Rabbit	Chicken
Human	37° C	eugonic	+	+	++++	=	-
Bovine	37° C.	dysgonic	-	-	++++	++++	-
Avian	40-42° C	rapid	+	+	+	++	++++

Pigs suffer from natural infection with both bovine and avian tubercle bacilli derived from infected cattle and poultry; they are also susceptible to infection with the human variety. In young pigs infection with bovine bacilli is generalized and acute with lesions in the lymphoid tissue, abdominal viscera and lungs. Infection with the human bacillus is generally localized, but the avian type may produce a generalized infection.

Other domestic animals suffer from tuberculosis to a considerably lesser extent. Horses, dogs and cats are occasionally infected, and the disease is rare in sheep and goats.

Experimental Animals. Experimental animals vary in their susceptibility to the varieties of the tubercle bacillus and in the type of infection produced. Of these animals only two need be considered here, the guinea pig and the rabbit. The guinea pig is highly susceptible to both bovine and human bacilli, and death follows the subcutaneous injection even of small doses in six to fifteen weeks. The lymphatic glands, spleen and liver are most affected, the lungs only slightly and the kidneys never. The necrotic areas in the spleen and liver are the most striking feature of the gross pathology and are peculiar

lymphogranuloma may be regarded as strong confirmatory evidence (Bedson 1950a). It may be added that the lymphogranuloma virus may be distinguished from other viruses in the group by the cross-neutralization test, but this procedure is cumbersome (Hilleman 1945, St. John and Gordon 1947).

Chemotherapy.—The lymphogranuloma-psittacosis group of viruses seem to be intermediate between the rickettsiae and the filtrable viruses in their susceptibility to chemotherapeutic agents. Both by egg culture and by animal experiment they exhibit some degree of susceptibility to the sulphonamides, the nitro-acridines, the quinoxalines, penicillin and chloramphenicol and still more to aureomycin and terramycin (see Rodaniche 1942, Hamre and Rake 1947, Hurst *et al* 1953). Clinical experience of some of these drugs in the treatment of the disease in man is rather disappointing (Kramer *et al*. 1944), though favourable reports have been made on aureomycin. How the antibiotics act is unknown, but they appear to suppress the growth of the virus rather than to destroy it. Relapses may therefore be expected owing to the survival of living virus in the tissues.

PSITTACOSIS

This is primarily a disease of birds, which sometimes attacks man. The first human case reported was by Ritter (1879-80) in Switzerland, who described the clinical and pathological features of the disease. Little attention was paid to the disease till 1929-30 when psittacosis appeared in many places throughout the world. In the United States alone 74 foci of infection were recognized, and 169 cases occurred with 33 deaths (Armstrong 1930). The incubation period in man is usually about 6-15 days. The disease resembles typhoid fever in many respects and is often complicated by pneumonia. Infection occurs by the inhalation of infective material excreted by parrots, parakeets, love-birds (budgerigars), canaries, or other birds. Case-to-case infection is rare. Workers in a laboratory where the virus is being handled are particularly prone to develop the disease (McCoy 1934).

For many years psittacosis was regarded as a disease affecting primarily birds of the parrot family; but in 1938, after an epidemic of pneumonia among the inhabitants of the Faroe Islands, widespread infection was demonstrated among the fulmar petrels (*Fulmarus glacialis*), which breed on the coast and are used by the inhabitants as food (Haagen and Mauer 1938, Rasmussen-Ejde 1938, Bedson 1940). Infection of human beings was four times as common in women as in men, and was apparently contracted by the inhalation of infected dust during the plucking of the birds' feathers. Later, Meyer and Eddie (1942) in the United States found that chickens were infected; and Meyer, Eddie and Yanamura (1942) showed that latent infection was present in pigeons collected from different parts of the States. Indirect evidence of infection of turkeys and ducks was brought by Eddie and Francis (1942). There is also reason to believe that latent infection may occur in cattle and sheep. In this country the isolation of the virus from pigeons was reported by Andrewes and Mills (1943). It is thus evident that psittacosis is a common avian infection. Usually it appears to remain latent, but under certain conditions it may give rise to clinical disease and prove fatal. Man is infected mainly from parrots and budgerigars, owing possibly to his closeness of contact with these birds.

to the guinea pig. True tubercles are seldom seen except in the very early stages of the disease.

Rabbits are highly susceptible to infection with the bovine bacillus, somewhat so to the avian bacillus and quite resistant to the human variety. Injection of bovine bacilli produces a generalized infection that terminates fatally in two to three months. On autopsy tubercles may be found in the spleen and liver, but the lesions are most marked in the lungs and kidneys and may even be confined to them. Very large doses of human bacilli (10 to 50 mg. intraperitoneally) may produce a progressive infection but never the acute

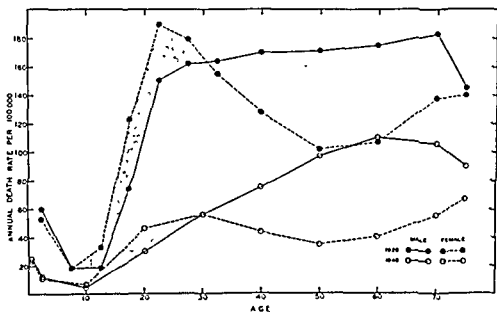


Fig. 149. The age and sex distribution of tuberculosis mortality (all forms) in 1920 and 1940. Note the differential incidence and decline over the twenty year period. If differentiation between white and colored rates is made for 1940, the picture is almost the same, i.e., the colored rates are very similar to the 1920 rates for both white and colored. Data from the reports of the Census Bureau.

fatal miliary disease. The mammalian tubercle bacilli, indistinguishable by cultural or serological methods, may, then, be sharply differentiated by their pathogenicity for these two experimental animals.

Neither rabbit or guinea pig is particularly susceptible to infection with the avian bacillus though avian tuberculosis may be produced in the rabbit.⁴¹ In the guinea pig death may be produced by large intraperitoneal doses, but on autopsy macroscopic tubercles are not visible, cultures and smears of the liver and spleen, however, show the presence of the bacilli. This form of tuberculosis, the proliferation of the bacilli without macroscopic tubercle formation, is known as the Yersin type of tuberculosis.

Epidemiology. In man tuberculosis is largely an airborne infection. Dissemination by milk is of relatively minor importance, particularly in the United States. The disease is one of civilization in that its transmission is

⁴¹ See Feldman *Avian Tuberculosis Infection*, Williams and Wilkins Co., Baltimore 1943.

of virus-like bodies in infected tissues; they grew these bodies in tissue culture; and they showed that they were specifically agglutinated by psittacosis antiserum, and that they contained two different antigens, one heat-labile and one heat-stable, both of which were demonstrable by the complement-fixation reaction. Microscopical examination of suitable infective material, such as the tissues of mice suffering from the disease, reveals the presence of minute coccal and bacillary bodies, arranged singly and in pairs, and bearing a resemblance to rickettsiae and to Paschen granules. They were described almost simultaneously by Levinthal (1930) in Germany, Coles (1930) in England, and Lillie (1930) in the United States, and are therefore sometimes referred to as L.C.L. bodies. They can be stained with Giemsa, and better still with River's modification of Castaneda's rickettsial stain. They can be thrown down almost completely by centrifugation at 5,000 r.p.m. for 2 hours. They are largely held back by a Berkefeld N, a Chamberland L2, or a Seitz EK filter, but go through a gradocol membrane having an average pore diameter of 0.6μ . Their probable size is about 200-300 $m\mu$ (Levinthal 1935, Lazarus *et al.* 1937). In tissue culture they increase rapidly in size, become embedded in a homogeneous ground substance, and are later replaced by smaller bodies (see Bland and Canti 1935). They can be cultivated on the chorio-allantoic membrane of the developing chick embryo for months without loss of virulence for mice, and can be consistently recovered from the spleen and liver of the embryo, which usually dies in 3-4 days (Burnet and Rountree 1935, Lazarus and Meyer 1939). They cannot be grown in the absence of living cells, nor do they appear able to grow extracellularly in tissue cultures (MacCallum 1936). They survive in frozen mouse spleen for 2 months, but die out rapidly in 50 per cent. glycerol phosphate at 6°C . Injected into guinea-pigs, they give rise to the production of agglutinins. The nature of these bodies is still in doubt, but in their general characteristics they would appear, as already pointed out, to be intermediate between the rickettsiae and the filtrable viruses (see Bedson and Gostling 1954).

Owing to minor differences in pathogenicity of virus strains isolated from different avian hosts, Meyer, Eddie and Yanamura (1942) suggested that the term psittacosis should be reserved for human infections contracted from psittacine birds, and the term ornithosis used for infections from petrels, pigeons and chickens. The wisdom of such a suggestion is open to doubt. If the practice should become established of coining a special name for the disease caused by every antigenic or pathogenic variety of an organism, then the whole nomenclature of the bacterial and virus infections will become unwieldy and confused.

Diagnosis and Immunity.—A rapid diagnostic test for the human disease, consisting of intraperitoneal injection of the patient's sputum into mice and the subsequent demonstration of elementary bodies in impression smears from the liver and spleen, was described by Rivers and Berry (1935). The virus may be present in the patient's blood during the first four days of illness. The serum of convalescent patients contains complement-fixing bodies, but has little neutralizing power (Bedson 1933). A positive complement-fixation reaction, which as already pointed out is not specific, may persist for years, possibly owing to the continued carriage of the virus. Post-mortem material should consist of portions of the lungs, any pleural or pericardial effusion, the liver and the spleen.

stration of virus in suspected birds is best accomplished by ...

facilitated by close contact. The frequency of positive tuberculin reactions rises rapidly from zero in the newborn to perhaps 90 per cent or over at puberty, and, as indicated earlier, almost all adults have been infected at one time or another. The infection is, then, widely disseminated through the human population. In most instances, however, clinical tuberculosis has not occurred and the lesions have healed. The proportion of persons with active clinical tuberculosis is not known; in some investigations the ratio of cases to deaths has been found to be as high as 10:1 or 12:1, but the ratio of reported cases to deaths is not much more than half of this. The prevalence of tuberculosis, then, defies precise definition.

There appear to be racial differences in susceptibility to tuberculosis. The death rate of the Negroes in this country is considerably higher than that of the white population, though the incidence of clinical tuberculosis in the two races is not greatly different. Whether this higher death rate is a consequence of environmental conditions or whether it is in part attributable to racial differences in susceptibility has been a point of considerable interest. It is probable that both factors are involved and that there is a real racial difference in susceptibility. In this connection the experience in the United States Army is of particular interest. Roth⁴² has found that in the years 1922-36 the average white morbidity rate was 2.10 per 1000 and the Negro rate 2.56, a ratio of 4:5; the white death rate was 0.24 and that of the Negroes 0.99, a ratio of 1:4; and the case-death ratios were 8.75 for the whites and 2.61 for the Negroes. Under the controlled conditions prevailing, i.e., preliminary physical examination, age and sex selection, the same housing conditions and identical diagnostic and therapeutic facilities, it would appear to be definitely established that though the incidence of clinical tuberculosis is no greater in the Negro, the disease is much more frequently fatal and the indicated greater susceptibility is a consequence of racial rather than environmental factors. Aronson⁴³ has reported similar findings, during World War II the Negro, while making up but 10 per cent of the United States Army, contributed 43.4 per cent of the total deaths from tuberculosis. There is some evidence that less well-defined races of man differ in their resistance to tuberculosis; Jews and Italians appear to be more resistant than the Irish.

The age and sex distribution of mortality from tuberculosis must be considered in relation to the decline in the disease rather than as isolated phenomena. That tuberculosis has been decreasing at a steady and relatively rapid rate since 1850 or thereabouts is indicated by the decline in the death rate from this disease. As shown in Fig. 150, the death rate from all forms of tuberculosis in the registration area of the United States has declined from 190.5 per 100,000 in 1900 to 40.1 in 1945, and a decline of this general order is apparent elsewhere. As in the case of some other diseases, the decline set in before the discovery of the bacterial etiology of infectious disease and the development of preventive measures, and hence is by no means entirely attributable to the practice of preventive medicine.

This decline has not been relatively the same in either the various age groups or in the two sexes. The death rate is highest in the very low age groups, that

⁴² Roth: Amer. Rev. Tuberc., 1938, 38:197.

⁴³ Aronson: Milit. Surgeon, 1946, 99:491.

is believed to be 2-3 weeks. The duration of the febrile disease is usually about 10 days. The initial rigor, the pleural pain, and the rusty sputum of pneumonia are seldom present, nor is there a leucocytosis. The disease does not spread readily by contact, it has no special seasonal incidence, and complications are uncommon.

Bacteriological studies have so far not been very illuminating. In a minority of cases the influenza virus, the psittacosis virus, the lymphocytic choriomeningitis virus, *Rickettsia burneti*, and even the fungus *Coccidioides immitis* have been found, but in most cases the findings have been negative. Several viruses giving rise to pneumonitis in small laboratory animals have been described; but with one or two exceptions, such as the virus isolated by Eaton and his colleagues (1944, 1945a) against which neutralizing antibodies were demonstrated in the patients' serum (Eaton *et al.* 1945b, Eaton and van Herick 1947), it is doubtful whether they were derived from the patients' sputum or were present in a latent form in the injected animals. Thomas and his colleagues (1945) suggested that a non-hæmolytic streptococcus—generally known as MG—might be responsible for some cases, because blood from the patients was found to agglutinate this organism; and the presence in other cases of cold agglutinins, quite distinct from the MG agglutinins, is indicative of a different ætiology. Experiments on human volunteers show that the disease can sometimes be transmitted by filtered sputa and throat washings (Report 1946), and is therefore probably due to a virus.

There is considerable variation of opinion among clinicians and pathologists on the nature and correct name of this disease. Some regard it as a clinical syndrome capable of being produced by a number of different agents. Others, on the contrary, believe it to be a specific disease caused by a particular, but hitherto unrecognized virus. The first group, on the whole, prefer the name primary atypical pneumonia. The second group consider it more logical to use the term virus pneumonia. There is no doubt that primary atypical pneumonia is an unsatisfactory term, and becomes still more unsatisfactory when, in reference to a particular case, it is preceded by the word typical. Bedson (1950b) objects to the term virus pneumonia, because some cases are of rickettsial and some of fungus origin. This merely means, of course, that *Rickettsia burneti* and *Coccidioides immitis*—and for that matter the tubercle bacillus—can produce a disease clinically resembling that which we are now considering. Obviously further knowledge is required before we can make up our mind on these difficult questions; but for our own part we feel that there is something to be said for both views. While admitting that the syndromes under discussion may be caused by a number of different agents, we consider it useful to define a disease which is usually caused by a primarily pneumotropic virus. For this particular disease we would use the term virus pneumonia, not denying of course that more than one primary pneumotropic virus may eventually be found responsible for it. It should be added that there is one extreme school of thought which denies the existence of a virus ætiology for the disease, and regards the syndrome as essentially an aspiration pneumonia following an infection of the upper respiratory tract (Robertson and Morle 1951).

The diagnosis of the disease is a clinical one, but in the laboratory attempts will be made to exclude known bacteria, rickettsiæ and viruses. In one-third to a half of the cases "cold" agglutinins are found reacting with human Group O red blood corpuscles at 0° C. but not at 37° C. (Turner 1943, Turner *et al.* 1943, Peterson *et al.* 1943, Horstmann and Tatlock 1943). These antibodies are absent in Q fever, but may be found in other diseases such as paroxysmal hæmoglobinuria, tropical

of one to two years, and falls rapidly in the five to nine group and then rises to a peak in early adult life, twenty to twenty-four, and declines with, in recent years, a small secondary peak between forty-five and fifty-four years (late adult tuberculosis). This secondary peak is a relatively recent development and is regarded by Frost⁴⁴ as a residual of higher rates in earlier life rather than a postponement of maximum risk. The decline in tuberculosis in the present century has been relatively greatest in the very young, an undoubted consequence of preventive measures.

The sex distribution of the death rates from tuberculosis in the various age groups is a curious phenomenon which has not been explained. The death rate for males of all ages is somewhat higher than that for females. In young adults, i.e., the fifteen to twenty-nine age group, the female death rate is considerably higher than the male death rate. In the higher age groups the male

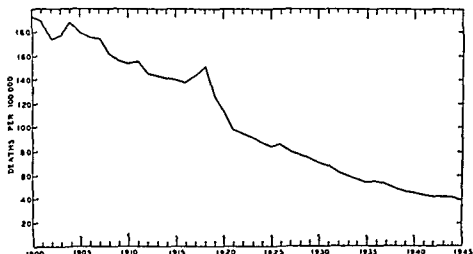


FIG. 150 The prevalence of tuberculosis, all forms, in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census

death rate becomes proportionately greater and exceeds the female rate for the rest of life. In the present century the female death rate in the higher age groups has declined somewhat more rapidly than the male death rate. The present status of the relation between the sex-specific death rates may be illustrated by the ratio of male to female deaths in the United States registration area in 1931-34 as given by Dauer.⁴⁵ For all ages the ratio of male to female deaths was 1.24, at ages under ten the ratio was 1.22, at ten to thirty years 0.69, at thirty to fifty five years 1.64, and at fifty five and over 1.57. (See Fig. 149)

Tuberculosis mortality has fallen at a more accelerated rate than deaths from all causes, in 1900 more than 11 per cent of all deaths were due to tuberculosis, in 1940 the percentage had fallen to 4.3. At the beginning of the century tuberculosis was first in numerical importance as a cause of death and it was seventh in 1940. Although the reported cases and deaths have de-

⁴⁴Frost *Amer. Jour. Hyg.*, Sec. A 1937, 3091.

⁴⁵Dauer *Amer. Rev. Tuberc.*, 1936, 37-435.

appears to be widespread in animals (Horsfall and Curnen 1946). When present in mouse lung suspensions heated to 80° C. for 10 minutes it is able to agglutinate mouse red blood cells (Mills and Dochez 1944, Curnen and Horsfall 1946). This property was used by Ginsberg and Horsfall (1951a) for following the growth cycle of the virus in the mouse's lung.

Eaton, Beck and Pearson (1941) isolated a virus from four cases of pneumonia. It proved virulent for mice by intranasal or intracerebral inoculation, but not by intraperitoneal inoculation. It did not pass through a Berkefeld V candle, and it appeared to be closely related to the meningo-pneumonitis virus, though not apparently identical with it antigenically (Beck and Eaton 1942). Elementary bodies were demonstrable in impression smears from infected organs.

Gönnert (1941) described a virus found in the lungs of normal mice. It proved highly virulent after intranasal mouse passage, giving rise to broncho-pneumonia. In the tissues inclusion bodies were numerous. Elementary bodies were visible in large numbers, particularly in the leucocytes. The virus, which is referred to as the *broncho-pneumonia virus*, apparently passed through coarse filters, and bore a close resemblance to the viruses of lymphogranuloma, psittacosis and trachoma.

Nigg and Eaton (1944) described a pneumotropic virus met with in normal mice. It proved highly virulent on intranasal inoculation, giving rise in small doses to focal lesions in the lungs, and in large doses to extensive consolidation. In the earliest stages of infection elementary bodies could be demonstrated in the large mononuclear cells. The virus had no affinity for the nervous system. It could be cultivated in the yolk sac of the developing chick embryo. It was pathogenic to mice and hamsters, but not to rabbits. By complement-fixation and cross-immunity tests it could be distinguished from other mouse pneumonitis and meningo-pneumonitis viruses. According to Rake, Shaffer and Thygeson (1942) it is susceptible to sulphonamides.

Eaton, Meiklejohn and van Herick (1944) (see also Eaton *et al.* 1945a, Eaton and van Herick 1947) were able to demonstrate a virus by inoculation of sputum or lung tissue from human patients into the amnion of the developing chick embryo. The virus is about 180-250 $m\mu$ in diameter. It is mildly virulent to hamsters and cotton-rats producing pulmonary consolidation. It is not adaptable to growth in the yolk sac or the extra-embryonic membranes of the chick embryo. Antibodies neutralizing the virus were found in over half the patients diagnosed clinically as primary atypical pneumonia.

Olson and Larson (1945) (see also Larson and Olson 1946) described a virus isolated from the blood and sputum during life and from the lung and spleen at post-mortem of patients suffering from a severe epidemic pneumonitis in Louisiana. The distinguishing features of this virus are its pathogenicity for guinea-pigs on intraperitoneal inoculation and for mice on either intramuscular or subcutaneous inoculation; both animals die within a few days. The virus is very pathogenic for cotton-rats, less so to white rats and hamsters. It can be grown in the yolk sac of the developing chick embryo. Elementary bodies staining by Macchiavello's method are present in the spleen of mice dying after intraperitoneal inoculation.

Nelson (1949) isolated a pneumotropic virus from wild rats in Princeton which he refers to as the *virus of wild rat pneumonia*. It was pathogenic to mice injected intranasally causing death in 7-14 days.

Andrewes and Glover (1945) described a pneumotropic virus, under the name *grey lung virus*, that is pathogenic for mice, cotton-rats and to a less extent other rodents. It rarely kills its host, but gives rise to extensive lung lesions that persist indefinitely (Andrewes and Niven 1950). No active immunity or antibody production can be demonstrated. Mice are susceptible by intranasal inoculation. The virus passes through a gradocol membrane of 450 $m\mu$, is destroyed by heating to 50° C in 30 minutes and by 0.3 per cent formaldehyde at room temperature in 24 hours. No inclusion or elementary bodies are found in the lung lesions. The classification of this virus is in doubt.

It may perhaps be mentioned that Sigurdsson, Grímsson and Pálsson (1952) in Iceland

clined markedly, there has not been any appreciable alteration in the proportion of cases to deaths, i.e., the case fatality rate.⁴⁶

It will be clear that the observed decline in tuberculosis is a complex matter for which there is no simple explanation. Although unknown factors are undoubtedly involved, it appears highly probable that the environmental improvements implied in the more decent living of the last few decades have contributed in no small part to the decrease in tuberculosis. The early detection and isolation of cases practiced in recent years has very likely had its effect also. Frost⁴⁷ has pointed out that the observed decline is indicative of the inability of the disease to reproduce itself; a surplus of chances to establish infection is, of course, essential and the required level of transmission may be very high and necessitate not only universal but frequently repeated exposure to relatively large doses of bacilli. If the requisite level is not maintained the disease must necessarily die out. In certain other diseases the causative agent may be disseminated by healthy persons; in tuberculosis, however, it is only the "open" clinical case that discharges bacilli into the adjacent environment. The present practice of isolation of active cases would appear, then, to be a sound one, and it is perhaps not unreasonable to hope for the eventual eradication of this disease.

THE BACILLUS OF LEPROSY (*MYCOBACTERIUM LEPRAE*)⁴⁸

Like tuberculosis, leprosy is an old disease of man. Perhaps more prevalent in ancient times, it is now most common in Central Africa, India, Japan and other Asiatic countries, and it was estimated that there were about 3,000,000 lepers in the world in 1924. The disease is prevalent in South America, with endemic centers in Brazil, Colombia and Argentina, and an overall incidence of more than 1 per 1000 population. Practically all the Caribbean islands are infected to about the same extent. It is relatively uncommon in Europe and sporadic cases occur in Latvia, Estonia, southern and eastern Russia, and on the Mediterranean coast.

Leprosy has been introduced into the United States with varying consequences. In Louisiana, Florida and Texas the imported cases have established foci in which the disease has a tendency to perpetuate itself, while in California and the central northwestern States it tends to die out. Elsewhere in the country transmission is so rare as to be negligible.⁴⁹ It is estimated that there are 500 to 1000 lepers in this country, many of whom are segregated in the National Leprosarium in Carville, Louisiana, the remainder living for the most part in California and New York.

Bacilli were found by Hansen in 1872 in the round epithelioid cells generally known as lepra cells, and the observation was one of the first of pathogenic bacteria.

Morphology and Staining. Morphologically the leprosy bacilli closely resemble the tubercle bacilli. They are long (6 μ), slender rods, usually straight, but sometimes slightly curved. They are non-motile and do not pro-

⁴⁶ Cf. Drolet. *Amer. Rev. Tuberc.*, 1938, 37:125.

⁴⁷ Frost. *Amer. Rev. Tuberc.*, 1935, 32:644.

⁴⁸ For a detailed consideration see Rogers and Murr: *Leprosy*. Williams & Wilkins Company, Baltimore 1940.

⁴⁹ McCoy: *Pub. Health Repts.*, 1942, 57:51.

swine influenza which, unlike virus pneumonia, is an acute disease of short duration with an incubation period of only 24-48 hours. Swine influenza moreover occurs mainly in the autumn and winter, whereas virus pneumonia shows little seasonal variation. It seems probable that virus pneumonia of pigs is caused by a virus belonging to the lymphogranuloma-psittacosis group, though no Castaneda-positive inclusion bodies have so far been recognized.

CAT DISTEMPER

A disease, known variously as distemper, nasal catarrh, or influenza, has been studied to a limited extent in the cat (Hindle and Findlay 1933, Dalling 1932, 1934, Baker 1912, 1914). It appears to be caused by a virus entirely different from that infecting dogs. The disease is highly infectious, debilitating, and lasts about a month. It is characterized by sneezing and coughing, and a mucopurulent discharge from the eyes and nose. Pneumonia is not usually detectable during life, but if the animal is killed, greyish densely consolidated areas are found in the anterior lobes of the lungs. The disease can be reproduced in cats by intranasal infection with ground-up pneumonic lung; parenteral inoculation causes only a mild fever. Mice injected intranasally die in 3-5 days, and after serial passage of virus in 2-3 days. Infection can be transmitted to rabbits, guinea-pigs, and hamsters as well by intranasal inoculation, but only mice and hamsters die. Films from the lungs of these animals show dense plaques and aggregations of elementary bodies similar to those seen in psittacosis. Baker's *feline pneumonitis* virus can be grown in the yolk sac of the developing chick embryo. It was purified by Moulder and Weiss (1951), who found the elementary bodies by electronmicroscopy to have an average diameter of 423 m μ . It is killed at 50° C. in 30 minutes. In 50 per cent glycerol it is inactivated within about a month. It is less pathogenic to mice than either the meningo-pneumonitis or the ornithosis virus. Its toxin is neutralized only by homologous antiserum (Hamre and Rake 1944). The serum of convalescent cats contains specific complement-fixing antibodies towards it.

Another virus isolated from feline pneumonia, but differing from Baker's virus, was described by Blake, Howard and Tatlock (1942). It may be noted that cases of pneumonia in human patients and in cats have not uncommonly been associated, though there is yet no definite evidence to show that the same virus was responsible for both.

TRACHOMA

Trachoma is a widespread disease pursuing a chronic course from early conjunctival inflammation to late hypertrophy and cicatrization. According to Bland (1944) the four most characteristic signs are: (1) inclusion bodies—described as "Lindner's initial bodies" and "von Prowazek-Halberstaedter inclusion bodies" (see Bengtson 1928) in conjunctival epithelial scrapings; (2) follicular reaction in the tarsus as well as in the fornices; (3) invasion of the cornea by blood vessels—pannus; and (4) subsequent scarring of the conjunctiva. The disease is peculiar to man and cannot be reproduced in its typical form in any of the lower animals. Monkeys suffer from a spontaneous follicular conjunctivitis which resembles trachoma in certain respects, and which has given rise to a great deal of confusion in the past. Grivet monkeys (*Lasiopyga griseoviridis*: syn *Cercopithecus athiops*) and vervet monkeys (*Lasiopyga pygerythra*: syn *Cercopithecus pygerythra*) can be

duce spores. They generally occur within the cells but are sometimes found free in the lymph spaces. Their arrangement within the cells is characteristic, several bacilli being usually grouped together in bundles like packets of cigarettes.

The staining reaction of these microorganisms is much like that of the tubercle bacilli. They stain somewhat more readily than the latter, and also decolorize more quickly with acids, but the difference is not great enough for differentiation. The presence of large numbers of bacilli within the cells, together with the clinical features of the disease, makes it possible to distinguish leprosy bacilli from tubercle bacilli without difficulty. Because of their acid-fast staining characteristic and their morphological resemblance to the tubercle bacilli and similar bacteria, these bacilli are included with the mycobacteria and designated *Mycobacterium leprae*.



Fig. 151 The leprosy bacillus. Acid fast stained smear from a skin lesion. Note the characteristic tendency to parallel arrangement of the bacilli in packets. $\times 1800$.

Cultivation. Numerous unsuccessful attempts to cultivate Hansen's bacillus on artificial media were made for years by bacteriologists all over the world. A few investigators have reported positive results. In most instances acid fast bacilli have been cultivated, but in others a variety of microorganisms, including diphtheroids, actinomycetes and anaerobic bacilli, have been found. It has been suggested by some workers that the acid fast bacilli present in leprosy lesions

body
organism

tained apparent growth and continued multiplication in subculture on an artificial medium of an acid fast bacillus. His results were confirmed by others, in particular Duval,⁵² who reported cultivating a bacterium regarded as *Mycobacterium leprae* directly from human tissue on artificial media. Acid fast bacilli, some

⁵⁰ Kedrowski: Ztschr. f. Hyg., 1901, 37:52.

⁵¹ Clegg: Philippine Jour. Sci., 1909, 4:403.

⁵² Duval: Jour. Exp. Med., 1910, 12:649.

(1938) show that inclusion blennorrhœa can be transferred to monkeys, especially baboons, and that, in contrast to experimental trachoma, inclusion bodies can be demonstrated in the conjunctiva of the inoculated animals. Elementary bodies about $0.25\ \mu$ in diameter are found in infective material; they can just pass a collodion filter of $0.7\ \mu$ A.P.D. Tissue cultures have so far been unsuccessful. The disease does not seem to be highly contagious. The natural habitat of the virus appears to be the genito-urinary tract, in which it gives rise to sub-clinical cervicitis in the female and non-specific urethritis in the male. Infection is transmitted venereally. The eyes of the infant become contaminated during birth. Unlike trachoma, ophthalmia in the new-born infant occurs during the first 12 days or so of life, and the demonstration of conjunctival inclusion bodies at this time is diagnostic of the disease. Inclusion blennorrhœa may be successfully treated by the topical application of sulphonamides in infants and by oral administration in adults. Aureomycin and terramycin are said to be of value given systemically. Chlorination of swimming baths is desirable to destroy the virus. (For reviews, see Thygeson 1918, Bedson *et al.* 1950.)

ENZOÛTIC ABORTION OF EWES

An enzoötic infectious disease causing abortion in ewes in the south-east of Scotland was described by Stamp, McEwen, Watt and Nisbet (1950). Premature lambing or abortion occurs during the last 3 or 4 weeks of pregnancy. Up to 30 per cent. of the ewes may be affected. Second abortions are uncommon in the same animal, suggesting that immunity develops. The cotyledons and foetal membranes are diseased, and can be shown microscopically to contain large numbers of elementary bodies staining by Macchiavello's and Castaneda's methods. The disease can be transmitted to pregnant ewes by subcutaneous or intravenous injection of a suspension of infected material. The virus can be cultivated in the yolk sac of the developing chick embryo and on the chorioallantoic membrane. It undergoes a cycle of development similar to that of the psittacosis virus (Stamp 1951). Its membership of the lymphogranuloma group is further shown by the possession of the same heat-stable antigen as that found in other members of the group. Psittacosis virus, for example, fixes complement in the presence of sera from infected sheep, and the sheep virus fixes complement in the presence of sera from human cases of psittacosis and lymphogranuloma (Barwell and Bishop 1951). Retrospective diagnosis of the disease in ewes can be made by the complement-fixation test; this becomes positive within 2 weeks of abortion and remains positive for at least 4 months (Stamp, Watt and Cockburn 1952). Natural transmission of infection probably occurs through contact with the diseased foetal membranes. The virus appears to remain latent in the tissues till the following pregnancy (McEwen *et al.* 1951). There is no evidence that the disease is tick-borne. Vaccination is said to reduce the incidence of abortion.

Sporadic Bovine Encephalomyelitis—This disease has been noted mainly in the Middle West of the United States of America. The clinical picture is one of fever, listlessness, gradual emaciation, watery discharge from the eyes and nose, spasm of muscle groups and lameness, weakness of the limbs, and eventual prostration. The course is 1-3 weeks, and 40-70 per cent. of affected animals die. At necropsy serofibrinous peritonitis and pleuritis are found, and there is histological evidence of meningo-encephalomyelitis. The disease is not primarily one of the nervous system. There is a widespread invasion of

of them chromogenic, have been cultivated by other workers, and there are at present a number of cultures in various laboratories labeled "*Myco. leprae*."

It seems highly probable that not any of these bacteria are leprosy bacilli in that they are etiologically related to the disease in man.⁵³ They are very likely best grouped with the saprophytic acid-fast forms such as the smegma and timothy bacilli.

The cultivation of the bacilli of leprosy has been intensively investigated in recent years by Soule and McKinley, who, in 1932, first isolated in culture a very slow-growing, non-chromogenic acid-fast bacillus which closely resembled the bacilli observed in leprous nodules. On media suitable for the growth of tubercle and other acid-fast bacilli incubated in an atmosphere of 40 per cent oxygen and 10 per cent carbon dioxide for six weeks, tiny, discrete colonies appeared. Although a number of isolations failed to grow on subculture, two strains have been maintained through 40 transfers over

RELATIVE PROPORTION OF TYPES OF LEPROSY*

Region	Neural Type	Lepromatous Type
Africa	90 5%	9.5%
Philippines	50 0%	50 0%
Mexico	40 0%	60.0%
Java	29 0%	71 0%

* Data from Lowe Proc. Sixth Pacific Sci. Congr., 1942, 5:921.

a period of six years.⁵⁴ These cultures were obtained from leprous material in Puerto Rico. Two years later Soule⁵⁵ reported that of 42 specimens obtained in the Philippines, 25 yielded cultures of the same or a very similar bacterium, and of these two strains were maintained on repeated subculture. The intradermal inoculation of rhesus and Cebus monkeys resulted in the production of temporary granulomatous lesions which could not be transmitted in series. Whether these bacilli are, in fact, leprosy bacilli is not clear; proof demands the reproduction of the disease, but since inoculation of experimental animals with relatively large amounts of macerated leprous nodules has not produced the disease, the negative results with cultures are, perhaps, not significant. More recently Loving⁵⁶ has reported the cultivation of the bacilli in a thiamine-enriched medium.

Pathogenicity. Although any organ or tissue in man may be attacked with varying results, two distinct types of leprosy are usually recognized—the nodular and the anesthetic. The former, which is the more acute, is char-

⁵³ Four strains of "leprosy bacilli" isolated in recent years were intensively studied at the National Institute of Health, but there was no evidence of a causal relation to the disease. Cf. Nat. Inst. Health Bull. No. 173, 1940.

⁵⁴ Soule and McKinley. Amer. Jour. Trop. Med., 1932, 12:1, 441, Jour. Amer. Med. Assn., 1932, 98:361; Amer. Assn. Advancmt Sci., Symposium Ser., 1938, 1:87; McKinley: Int. Jour. Lepr., 1939, 7:1, 217.

⁵⁵ Soule Proc. Soc. Exp. Biol. Med., 1934, 31:1197.

⁵⁶ Loving Amer. Jour. Trop. Med., 1943, 23:593.

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acterized by the development of masses of granulation tissue, the so-called "leproma," which may appear superficially in different parts of the body, and by their growth and coalescence produce distortion and mutilation. The anesthetic type, or nerve leprosy, progresses more slowly, the average duration of the cases being nearly twice as long (eighteen years) as cases of the nodular type, some being known to extend over thirty-five or forty years. Atrophy of the muscles and other trophic disturbances accompany the nerve lesions. Very many lepers die of other diseases, Kean and Childress,⁵⁷ for example, reported that, of a group of 82 lepers autopsied, 24 died of tuberculosis, 22 of neuritis, 15 of leprosy, 10 of heart disease, 4 of cancer, and the remainder of miscellaneous diseases other than leprosy.

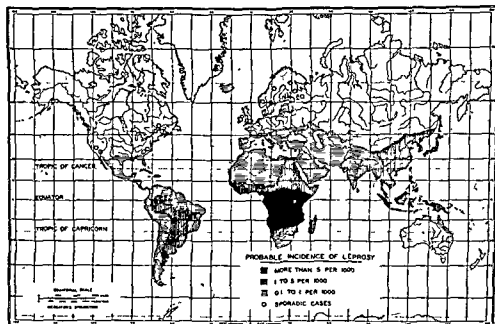


Fig. 152. The probable prevalence of leprosy in the world as indicated by present data. Based on Goode Base Map No 201 M. By permission of the University of Chicago Press. (After Saunders)

In both forms of leprosy Hansen's bacillus is found in all cases, in enormous numbers as a rule in the lesions of nodular leprosy, and less abundantly in the anesthetic type. Very few bacilli are observed outside the body cells, and they are found in the cytoplasm and do not invade the nucleus. Almost any part of the body may be the site of leprosy growth, the kidneys are usually invaded, the liver and spleen always. The bacilli have been seen in the central nervous system and are occasionally found in the blood, generally in the leucocytes but sometimes free.

Leprosy has never been produced in experimental animals by the inoculation of leprosy material from man, even the implantation of nodules containing enormous numbers of bacilli has been without effect.

⁵⁷ Kean and Childress: *Internat. Jour. Leprosy*, 1942, 10 51.

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Transmission. Leprosy is probably transmitted by contact, though the conditions that make transmission possible are entirely unknown. There are numerous instances in which healthy persons, such as asylum attendants, have been more or less in contact with lepers for long periods without contracting the disease. In other cases, however, leprosy has been contracted by those in close and long-continued contact with diseased individuals. There is also indirect evidence of transmission. Manson⁵⁸ cites the case of an Irishman who acquired leprosy in the West Indies. On his return to Ireland his bed was shared by his brother who, moreover, sometimes wore the leper's clothes. The brother, who had never been in any foreign country, became in time an undoubted leper. In this instance communication from one person to another was practically demonstrated. Currie⁵⁹ found that a large percentage of cases studied in Hawaii gave a history of exposure, and that usually such exposure was of an intimate character. Saunders⁶⁰ has pointed out that leprosy occurs for the most part in populations of low social order living under unsanitary conditions of poverty, crowding, uncleanness and sickness. Even so a relatively small proportion of those exposed develop the disease. Doull *et al.*⁶¹ have shown that age at the time of exposure is important, the risk being greatest for those exposed before five years of age. The average interval between exposure and development of leprosy in their study was 10.5 years for those exposed under ten years of age, and 6 years for those exposed over ten years of age.

One way in which the bacillus may leave the body is in the nasal mucus, and large numbers of the bacilli are found in the secretions of the nose in many cases. Bacilli may sometimes be discharged from the mouth or nose in small particles of mucus. In the opinion of many writers, the mucous membrane of the nasopharynx is the point at which the bacteria are introduced into the body, as well as the chief source from which infection is spread.

Evidence of the direct inoculability of leprosy from man to man is quite inadequate. Many attempts to infect healthy persons have been made and have failed, and one often-cited instance of successful inoculation is by no means unimpeachable. In the case of the criminal Keanu in the Hawaiian Islands, reported by Arning,⁶² implantation of material from a leprosy nodule was followed by the development of true leprosy, which terminated fatally six years after inoculation. The experiment, however, did not include the important source of error involved in the facts that Keanu was a native of a country in which leprosy was common, that he had lived among lepers, and that members of his family were lepers. More recently Lazoudaky⁶³ inoculated himself intramuscularly with blood from two lepers and contracted the disease.

Much light is thrown on the contagious character of leprosy by the success that has attended the isolation and segregation of leprosy patients. The Norwegian experience showed that a careful but not unduly rigorous system of

⁵⁸ Manson. *Tropical Medicine*. London. 1900, p. 448.

⁵⁹ Currie. *Pub. Health Bull.* No. 41, 1910.

⁶⁰ Saunders. *Proc. Sixth Pacific Sci. Congr.*, 1942, 5:957.

⁶¹ Doull, Guinto, Rodrigues and Bancroft. *Amer. Jour. Trop. Med.*, 1945, 25:435.

⁶² Arning. *Arch. f. path. Anat.*, 1893, 134:319.

⁶³ Lazoudaky. *Jour. Trop. Med. Hyg.*, 1937, 40:77.

scabs of the skin lesions may likewise prove infectious; laundry workers are sometimes infected in this way.

According to Downie (1951a), the virus enters by the respiratory tract and produces a minimal or closed non-infectious lesion in the mucosa. Thence it passes to the lymphatic nodes and by the blood stream to the internal organs. Progressive infection of cells in these organs takes place with corresponding increase in the amount of virus. Towards the end of the incubation period the virus overflows into the blood stream producing a secondary viraemia. The skin and other tissues become infected, and the formation of antibody follows a few days later. The maturation of the eruption proceeds after the antibody has appeared, as the virus is already in the cells and is protected from the antibody.

Animal Inoculation.—Smallpox cannot be reproduced in its typical form in any of the lower animals. In calves and rabbits only minimal lesions are produced; and if material from these lesions is passed continuously through a series of animals, the virus is so altered that it gives rise to vaccinia, and reproduces this type of infection when again inoculated into a child.

The most susceptible animal to *variola* is the monkey. Intracutaneous inoculation of pus from a human patient gives rise to a papule after 5 days; by the 8th day this is vesicular, and by the 10th day a typical pock is formed, with a raised congested edge surrounding a crateriform depression, partly filled with dark purulent material (Blaxall 1923, Gordon 1925). Intratesticular inoculation of the monkey with *variola* virus gives rise to an orchitis with the appearance of a rash on the scrotum. Passage from one monkey to another is almost uniformly successful. Cats and dogs are said to be less susceptible than monkeys, but more so than rabbits. Infection in the less susceptible animals may be of the "inapparent" type, in which immunity develops in the absence of any clinical manifestations of disease (Teissier *et al.* 1931). Pus from cases of alastrim gives rise to very much the same lesions as that from the classical type of smallpox.

Both calves and rabbits can be readily infected with *vaccinia*. Inoculation of the scarified skin of a rabbit with calf lymph causes a red swelling on the 3rd day, which increases until a red papule is formed, surrounded by a red areola; the papules become vesiculated about the 5th day, and tend to coalesce and to become pustular; scabbing follows on the 7th or 8th day. A good calf lymph should give a reaction in the rabbit when diluted 1/1000; sometimes a reaction occurs even in a 1/100,000 dilution. If vaccine lymph is inoculated intravenously into a rabbit, and a portion of the skin is thereafter shaved, vaccinal papules appear about the 3rd day on the shaved area (Calmette and Guérin 1901). In poorly nourished and in nursing rabbits the susceptibility of the skin to infection is said to be decreased (Sprunt 1942, Pearce *et al.* 1936 a, b). If a very large dose of vaccine lymph is inoculated intravenously into a rabbit—1 to 2 ml. of undiluted stock emulsion—or a highly virulent virus is employed, a generalized eruption occurs over the entire body surface, and lesions may develop in the internal organs (Noguchi 1918, Douglas *et al.* 1929, Armstrong and Lillie 1929). Subcutaneous inoculation may give rise to a local pustular eruption, but often no superficial lesion appears at all. Intratesticular inoculation results in the development of an orchitis and oedema of the scrotum; the animal dies about the 6th day. Inoculation on the scarified cornea gives rise to a keratitis and purulent conjunctivitis. Intracerebral inoculation of rabbits is followed by trembling, paralytic, and other nervous symptoms and by death on the 5th to the 8th day; the brain and spinal cord of these animals prove infective to fresh rabbits, when inoculated on to the cornea or intracerebrally (Marie 1920). After intranasal inoculation, the virus can be demonstrated in the cervical lymph glands in about 12 hours; it appears to become attached to the lymphocytes and hence finds its way into the blood stream (Yoffey and Sullivan 1939).

In mice a vesiculo-pustular reaction may often be obtained by inoculation of the scarified foot pad or tail with vaccinia virus (Rosenau and Andervont 1931).

separation was accompanied by a diminution of the number of cases from 2870 in 1856 to 577 in 1900. The circumstance that infection does not invariably follow chance contact or association should not, therefore, lead to neglect of the facts that leprosy is a bacterial disease; that up to the present under natural conditions the specific bacterium has not been found except in the human body; and that, so far as is definitely known, the leper himself is the most important means by which leprosy spreads.

Immunity. Man is clearly highly resistant to infection with the leprosy bacillus, and, after infection is established, the ensuing disease is essentially a benign process that may take many years to terminate fatally, in fact, many lepers die from other causes. Little is known regarding the specific immune response. Lepers do, however, develop a hypersensitivity to the cell substance of acid fast bacilli such as the tubercle bacillus and the various saprophytic species. A curious fact is the appearance of a positive Wassermann reaction in lepers in the absence of syphilis. According to some, this is an artifact arising from infection with yaws, and it is said that the Kahn test is negative in non-syphilitic lepers. Recent investigations,⁶⁴ however, have shown that both the Wassermann and the Kahn tests may be positive in lepers having no clinical symptoms of syphilis.

Lepromin. The hypersensitivity developed by the leper may be demonstrated by the intradermal inoculation of material prepared from leprosy nodules. Two reactions are observed, one early and occurring after three or four days, and the late reaction appearing three to four weeks after inoculation. The reaction is not specific in that a response is also elicited by preparations of other acid fast bacilli. Dharmendra⁶⁵ has isolated a nucleo protein from leprosy nodule material, however, which appears to have some specificity. In its present form,

Rat Leprosy. Leprosy is also known as rat leprosy, and characterized by enormous numbers of acid fast bacilli present in the lesions, was described by Stefansky in 1903, as occurring in wild rats in Odessa. The disease was observed in the same year by Dean, who later showed it to be transmissible. It has since been observed in wild rats all over the world.⁶⁶

The acid fast bacilli closely resemble the leprosy bacillus in size and shape and are found intracellularly but not as often in the packet arrangement of parallel bacilli. *Myc. leprae murium* has not been cultivated on artificial media. The disease may, however, be transmitted to white rats, mice and guinea pigs by inoculation with pieces of tissue.⁶⁷ The experimental infection is a relatively benign process. A local, circumscribed lesion develops following subcutaneous inoculation which becomes palpable in four to five weeks and eventually develops to a large tumorous mass ulcerating on the surface and

⁶⁴ Badger, Patrick, Fite and Wolfe in Nat. Inst. Health Bull. No. 173, 1940, Faget and Ross. Ven. Dis. Information, 1944, 25 133.

⁶⁵ Reviewed by Dharmendra and Lowe. Leprosy Rev., 1946, 17 9.

⁶⁶ See the review by Lowe. Int. Jour. Lepr., 1937, 5 311, 463, also Fielding. Med. Jour. Australia, 1945, 32 473.

⁶⁷ For recent studies on experimental rat leprosy see Fite in Nat. Inst. Health Bull. No. 173, 1940.

granules. These Guarnieri corpuscles may be produced very rapidly—in a few hours—by inoculation of the scarified cornea of a rabbit with the vaccinia virus. Fixed, and stained with methylene blue and eosin, they appear as irregular pink and lilac masses, which indent the nuclei, and which may contain acidophilic or

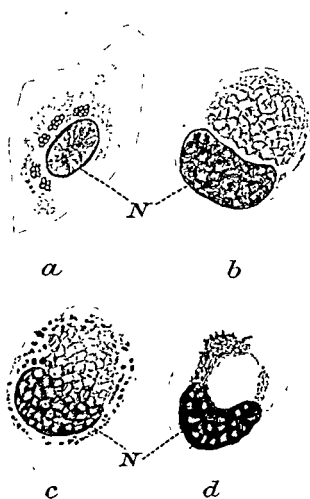


FIG. 301.

Guarnieri corpuscles in the cornea of an experimentally inoculated rat.

- a* = five small vaccine bodies in congeries.
- b* = fully developed vaccine body.
- c* = vaccine body showing reticulum breaking up into granules; similar granules throughout cytoplasm.
- d* = vaccine body with central portion consisting of homogeneous material.

N = nucleus.

(After Ewing.)

pustules are as a rule bacteriologically sterile (Mair and Parker 1953).

The virus of variola is not easily filtrable when present in tissue extracts, probably because of the imperfect liberation of the virus from the cells or its adsorption by particulate matter in the suspension (see Green and Eagles 1931). The filtrability of the elementary bodies in vaccine lymph was demonstrated by Negri (1906). The cultivation of variola and vaccinia viruses in the rabbit's testicle, in tissue cultures *in vitro*, and on the chorio-allantoic membrane of the developing chick embryo were described in Chapter 41, and are referred to again on p. 2115.

basophilic granules (Figs. 300, 301). Besides the Guarnieri corpuscles, which are always present at some stage in variolous and vaccinal lesions, very tiny intracellular bodies, 0.1–0.25 μ in diameter, have been described; they are generally called "Paschen's granules" (see Fig. 235, p. 1086). They were first observed by John Buist of Edinburgh in 1887 (see Gordon 1937). The work of Ledingham and his colleagues, of Paschen, and of others (see Chapter 41) leaves no doubt that these granules, or elementary bodies as they are often called, constitute the actual causative virus of the disease. The Guarnieri corpuscles probably represent intracellular microcolonies of the virus. The microscopical demonstration of the virus particles *in situ* may be accomplished by suitable staining methods (see Taniguchi *et al.* 1932*a*, Haagen and Kodama 1934, Herzberg 1934, 1936, Tang and Wei 1937). For a description of the general properties of vaccinal elementary bodies, the reader is referred to the review by Smadel and Hoagland (1942), and for their morphology under the electron microscope in sections of infected tissues to a beautifully illustrated paper by Morgan and his colleagues (1954). It should be noted that the contents of smallpox vesicles and

persisting throughout the life of the rat. The earliest lesions in the other organs do not appear before four to six months, and the animal dies only after a year or more.

The relation of rat leprosy to human leprosy is not known. Rats are not susceptible to inoculation with human leprosy material.

OTHER ACID-FAST BACILLI

Mycobacterium Paratuberculosis.⁶⁸ A chronic enteritis of cattle usually terminating fatally is caused by an acid-fast bacillus closely resembling the avian variety of the tubercle bacillus. The disease is sometimes called *Johne's disease* and the bacillus *Johne's bacillus* after its discoverer. The disease only remotely resembles tuberculous infection. The lesions in the intestinal wall are proliferative and the granulomatous tissue may contain epithelioid cells and occasionally giant cells, but there is no caseation.

The disease appears to be widespread in the United States. Infected cattle become hypersensitive to the bacillary substance and filtrates of cultures produce a skin reaction analogous to the tuberculin reaction which is designated the "johnin reaction." No case of human infection with *Myco. paratuberculosis* has been recorded.

The Vole Bacillus. An acid-fast bacillus responsible for an epizootic, chronic infection of the field vole, *Microtus agrestis*, resembling tuberculosis was discovered by Wells⁶⁹ in 1937. It closely resembles the tubercle bacillus culturally though it forms no pigment and growth is not enhanced by glycerol. It is pathogenic for both guinea pigs and rabbits, considerably more so for the latter, and is not pathogenic for fowls. Brooke⁷⁰ has suggested that it is a distinct type of mammalian tubercle bacillus and should be called *Mycobacterium tuberculosis* var. *muris*. This microorganism has been of particular interest because, though it produces only a localized and retrogressive infection when inoculated in small doses in guinea pigs and calves, tuberculin sensitivity is produced, and preliminary experiments on its use as a prophylactic have given suggestive results.

The "Cold-Blooded" Mycobacteria. Acid-fast bacilli have been found associated with pathologic processes in various cold-blooded animals. In some instances the processes superficially resemble tuberculous lesions. *Myco. piscium* was isolated from nodules and tumor-like formations in carp; *Myco. marinum* from "tuberculosis" of sea bass and certain other salt-water fish; *Myco. ranarum* was found in the liver of a frog; *Myco. thamnophaeos* is a parasite in garter snakes, and *Myco. chelonae*, the "turtle bacillus," has been referred to above.

The saprophytic acid-fast bacilli include the well-known timothy bacillus, *Myco. phlei*, found in soil, on grasses and elsewhere in nature; the "butter bacillus," *Myco. butyricum*; and *Myco. smegmatis*, which is, however, a parasite found in both male and female smegma. The smegma bacillus is often difficult to distinguish from the tubercle bacillus on morphological grounds, and confusion of the two may have considerable practical importance

⁶⁸ Hagen and Thomson. Tr. Nat. Tuberc. Assn., 1931, p. 232.

⁶⁹ See the general review by Wells: Med. Res. Council Spec. Rept. Ser. No. 259, 1946.

⁷⁰ Brooke. Amer. Rev. Tuberc., 1941, 43: 806.

is mixed with an antivaccinial serum and incubated at 37° C., a finely floccular precipitate appears similar to that obtained with vaccinia material (Gordon 1925). Using this method, Tulloch (1928) was able to make a rapid diagnosis of smallpox during an epidemic in Dundee. For a description of the detailed technique reference should be made to the report by Craigie and Tulloch (1931). A more delicate method than precipitation is afforded by the complement-fixation test (Craigie and Wishart 1936). The contents of at least 6 vesicles or pustules, or the crusts from at least 6 lesions, are required. The test is more rapid than that of cultivation, being completed within 24 hours. It cannot however be used in the papular stage of the eruption, nor does it distinguish between variola and vaccinia. In severe cases of the disease with a fatal prognosis antigen may be demonstrated in the patient's blood (Downie *et al.* 1953).

(d) *Demonstration of antibody in the patient's serum.*—Antibody is most conveniently detected by the complement-fixation test using an antigen made with vaccinia or variolous lesions. The test does not become positive till the beginning of the 2nd week of the disease; it does not distinguish between variola and vaccinia, and it is unsuitable for use in persons vaccinated within the previous 6 or 12 months owing to the presence of residual antivaccinial antibody. Its chief value is in the diagnosis of atypical cases at a late stage of the disease, or during convalescence when the skin lesions have healed. Complement-fixing and anti-haemagglutinating bodies do not persist for more than 12 months after an attack of variola, though neutralizing antibodies may be demonstrated for years (Downie 1951a).

Immunity.

Our knowledge of immunity may be said to have started with Jenner's discovery in 1796 that inoculation with cow-pox protects against subsequent inoculation with smallpox; though it was known before that one attack of smallpox, naturally or experimentally incurred, protected against a second attack. Jenner believed that cow-pox was really smallpox, modified by passage through the cow—a belief rendered rather doubtful by investigations of recent years. Cutaneous inoculation of the calf with variolous material from man produces insignificant lesions; but if the material from these lesions is passed through further calves *in series*, then after three or more passages a good vesiculo-pustular eruption appears on the inoculated skin. This experimentally produced disease is similar to though apparently not identical with the naturally occurring cow-pox. If the pustules are scraped at the height of their development, ground up in a mortar, and mixed with glycerolated saline, the resulting product is indistinguishable except serologically from vaccine lymph. In practice it is found beneficial to pass the vaccinia virus from the calf to a rabbit, from the rabbit to a calf, from the calf to a child, and from the child back to a calf, or through some similar series; in this way the activity of the virus seems to be better maintained than by continuous passage through one species of host.

Experimentally, rabbits that have been inoculated with vaccinia virus, either cutaneously, subcutaneously, or intravenously, become resistant to a second inoculation within about 10 days. When repeated injections are given, the rabbits become hyperimmunized, and in their serum neutralizing viricidal antibodies can be demonstrated, as well as agglutinins, precipitins, complement-fixing and anti-haemagglutinating bodies (Ledingham 1924, Gordon 1925, Sobernheim 1925, Chu 1948). W. Smith (1929) brought evidence to show that, in rabbits inoculated

in the diagnosis of suspected cases of tuberculous infection of the urinary tract. It is, it may be noted, also found in the urine and may contaminate fecal specimens. The saprophytic bacilli all grow much more rapidly than the tubercle bacilli, and neither they nor the bacilli isolated from cold blooded animals are pathogenic for guinea pigs and rabbits, or at best only feebly so. The interrelationships of these non pathogenic acid fast bacilli are considered at length by Gordon⁷¹ and Gordon and Hagan.⁷²

⁷¹ Gordon Jour. Bact., 1937, 34-617.

⁷² Gordon and Hagan Jour. Bact., 1938, 36-39.